MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED TO SOFT IONIZATION TECHNIQUES: ANALYSIS OF TELECHELIC POLYMERS

DISSERTATION

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"Like light rays in the spectrum, the different components of a pigment mixture, obeying a law, are resolved on the calcium carbonate column and then can be qualitatively and quantitatively determined. I call such a preparation a chromatogram and the corresponding method the chromatographic method."

M. S. Tswett (1906)

ABSTRACT

Tailor-made macromolecules providing end group functionality, different chain topologies and controlled molar mass distribution raised enormous challenges to conventional characterization approaches. These analytical challenges initiated the necessity to develop multidimensional methodologies based on chromatographic as well as spectroscopic and spectrometric techniques to put up a better understanding on the structure-property relationship of complex macromolecules.

The present thesis demonstrates a simple combination of chromatographic methodologies based on one- and two-dimensional systems, which help to identify the functionality type as well as the molar mass distribution of synthetic oligomers and polymers, instantly. Thus, different synthetic aspects such as the efficiencies of the end group modification of amino oligomeric resins and poly(ε-caprolactone)s as well as the preparation of symmetrical/nonsymmetrical triblock copolymers were quantitatively analyzed by one- or two-dimensional LC/SEC coupled to soft ionization mass spectrometric techniques. The straightforward online coupling to electrospary ionization (ESI) source equipped with a time of flight analyzer (TOF) allowed a clear identification of the chemical composition of the investigated samples.

A combination of two-dimensional liquid chromatography coupled offline to a matrix-assisted laser desorption/ionization (MALDI) equipped with a time of flight analyzer (TOF) via a polymer fractionation interface module confirmed besides the chain topology and structural heterogeneity of copolymers also their multistep synthetic pathway. The presented combination of multidimensional chromatography with soft ionization mass spectrometry techniques (2D-LC/SEC-MS) is the technique of choice to fully identify the chemical heterogeneities and purity of polymeric samples in terms of their desired applications.

KURZFASSUNG

Maßgeschneiderte Makromoleküle mit speziellen Endgruppen-Funktionalitäten, Kettentopologien und kontrollierten Molekulargewichtsverteilungen stellen große Herausforderungen für herkömmliche Charakterisierungsmethoden dar. Diese analytischen Herausforderungen leiteten die Notwendigkeit ein, multidimensionalle Methoden basierend auf chromatographischen, spektroskopischen sowie spektrometrischen Techniken zu entwickeln und vor allem mit einander zu kombinieren, um ein besseres Verständnis über die Struktur-Eigenschaftsbeziehungen von komplexen Makromolekülen zu erhalten.

Die vorliegende Arbeit zeigt eine Kombination von analytischen Methoden auf Basis von chromatographischen und spekrtometrischen Techniken, die eindeutig die Funktionalitätsverteilung sowie die Molekulargewichtsverteilung von synthetischen Oligomer- und Polymerverbindungen bestimmen kann unter Verwendung einer eigenständig entwickelten Ein- oder Zwei-dimensionalen LC/SEC Kopplung mit massenspektrometrischen Techniken. Somit werden verschiedene synthetische Aspekte quantitativ erfasst, wie z.B. die Effizienz der Endgruppenmodifizierung von oligomeren Harzen und von Poly (ε-caprolacton)en oder die Reinheit von symmetrischen oder unsymmetrischen Triblockcopolymeren bestehend aus Poly(ethylenoxid) und Polyisobutylen.

Eine einfache Online-Kopplung von LC mit ESI-TOF Massenspektrometrie ermöglicht eine eindeutige Identifizierung der chemischen Zusammensetzung der untersuchten Proben. Wohingegen die Kombination von Zwei-dimensionaler Flüssigkeitschromatographie (LC/SEC) gekoppelt offline mit MALDI-TOF Massenspektrometrie über einen Fraktionssammler erlaubt die Identifizierung der strukturellen Heterogenität von Copolymeren, ihre Reinheit sowie ihre exakte Blocklängenzusammensetzung. Die vorgestellten Kombinationen multidimensionaler Chromatographie (2D-LC/SEC-MS) gekoppelt mit sanften Techniken der Massenspektrometrie ist die Methode der Wahl, um die chemische Heterogenität und Reinheit von Polymerproben vollständig zu untersuchen in Hinblick auf ihre gewünschten Anwendungen.

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ABBREVIATIONS

INSTRUMENTATION

^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR	Proton-1Nuclear Magnetic Resonance
2D-LC/SEC-MS	Two-dimensional liquid chromatography coupled to size exclusion chromatography combined with mass spectrometry
CE	Capillary electrophoresis
CEC	Capillary electrokinetic chromatography
DVISC	Differential viscometry
ELSD	Evaporative light scattering detector
ESI-TOF MS	Electrospray ionization – time of flight mass spectrometry
FFF	Field flow fractionation
GC	Gas chromatography
GPEC	Gradient polymer elution chromatography
HPLC	High performance liquid chromatography
IR	Infrared spectroscopy
LALLS	Low angel light scattering
LC/SEC	Liquid chromatography coupled to size exclusion chromatography
LS	Light scattering
MALDI-TOF MS	Matrix assisted laser desorption ionization – time of flight mass spectrometry
MALS	Multi angel light scattering
PDA	Photodiode array detector
QELS	Quasi-elastic (dynamic) light scattering
RALS	Right angel light scattering
RI	Refractive Index
SEC	Size exclusion chromatography
TDA	Triple detection detector
TGIC	Temperature gradient interaction chromatogr.
TREF	Temperature rising elution fractionation
UV	Ultraviolet spectroscopy
VISC	Viscometry

PHYSICAL TERMS

a	Mark-Houwink exponent
f	Molecular functionality
K_d	Distribution coefficient
k'	Dimensionless retention factor
ΔG	Gibbs free energy
ΔH	Interaction enthalpy
R	Universal constant
ΔS	Conformational entropy
T	Temperature

CHROMATOGRAPHIC TERMS

CAP	Critical adsorption point
CC	Critical conditions
IC	Interaction chromatography
LAC	Liquid adsorption chromatography
LCCC	Liquid chromatography at critical conditions
NP	Normal phase
NUC-NH_2	Normal phase column with aminopropyl functionality
NUC-OH	Normal phase column with hydroxyl functionality
O	Degree of orthogonality
P	Polarity index
RP	Reversed phase
R_t	Retention time in minutes
v/v	Volume fraction
V_0	Void volume
V_i	Interstitial volume
V_e	Elution volume
V_p	Volume of the pore region
w/w	Weight fraction

SOLVENTS

ACN	Acetonitrile
DCM	Dichloromethane
HFIP	Hexafluoroisopropanol
MEK	Methyl eythyl ketone
MeOH	Methanol
MTBE	Methyl tert-butyl ether
THF	Tetrahydrofuran

Polymeric Terms

CCD	Chemical composition distribution
EPC	Ethylene propylene copolymer
FTD	Functionality type distribution
MAD	Molecular architectural distribution
MMD	Molar mass distribution
M_n	Number-average molar mass
M_w	Weight-average molar mass
M_z	Z-average molar mass
n	Degree of polymerization
PB	Polybutylene
PBA	Poly(butyl acrylate)
PCL	Poly(ε-caprolactone)
PEMA	Poly(ethyl methacrylate)
PDI	Polydispersity index
PEO	Polyethylene oxide
PI	Polyisoprene
PIB	Polyisobuytylene
PMMA	Poly(methyl methacrylate)
PS	Polystyrene
PtBMA	Poly(t-butyl methacrylate)
PVAc	Polyvinyl acetate
PVP	Polyvinylpyrrolidone

To my family

INTRODUCTION 1

Polymeric materials based on complex polymer mixtures are a key role in our daily life applications.[1-8] As we enter the twenty-first century, countless numbers of applications of multi-component systems found their way to success. The chemical nature and structural features (chain topology or end group functionality) of polymers that affect their physical properties need to be well characterized in view of promising applications.[9]

Structural characteristics of polymers are basically influenced by their size (molar mass), which varies between broad and narrow molar mass distributions, depending on the synthetic pathway and primary conditions. Branching and chemical composition that forms the architectural distribution of polymers, *e.g.* graft, star-, brush- and comb-like polymers strongly determine the behavior of the polymeric materials.[10] Furthermore, the molecular arrangement of monomeric units within copolymer chains *e.g.* alternating, statistical or block copolymers, affect their morphological distribution together with the physico-chemical parameters.[9, 11, 12] Therefore, complex polymers may exhibit more than one distribution distinguished by the molar mass distribution (MMD), the chemical composition distribution (CCD), the molecular architecture distribution (MAD) and functionality type distribution (FTD). These molecular heterogeneities are considered significant as they shape the future application of macromolecules, triggering the investigation of structure-property relationships by a large variety of analytical techniques.[13]

Traditional methods such as infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR) are proved to be beneficial for the determination of type and composition of monomers as well as the identification of functional end groups within homo- and copolymers.[11, 14] However, it is quite tedious to analyze end groups or special linkages within the polymer chains, which are small in their weight fractions compared to the polymer backbone, alongside with the lack of information concerning the monomeric and/or functional distribution of the investigated molecules.[15]

Herein, separation science is a useful path for the determination of the different polymeric distributions, as mentioned above. A particular field is the size-exclusion chromatography (*SEC*), which provides information about the molar mass distribution (MMD) by calculating the molar mass averages (M_n, M_w and M_z) of polymer molecules.[16]

Other chromatographic techniques such as liquid adsorption chromatography have been developed to inspect the chemical heterogeneity of macromolecules on the basis of free Gibbs energy,[17] which influences the interaction of a single chain with the stationary phase.[18, 19]

As the complexity of macromolecules illustrated by the tremendous plethora of synthetic routes increases,[20] chromatographic methods using exclusion principles (*SEC*) or interaction chromatography (IC), would not solely reveal the purpose of analysis. Consequently, combining these or even similar methods into a multidimensional system can enhance the resolving power over one-dimensional counterparts. The applicability of a multidimensional system is affected by the degree of orthogonality of chromatographic modes (dissimilarity in function) that governs the separation efficiency.[21, 22] Online coupling techniques of different chromatographic devices are considered by far as efficient and reliable separation strategies with the possibility of automation via special interfaces.[23, 24]

Structural studies of unknown polymeric samples utilizing chromatographic separation modes is a relatively complicated task and the correlation of chromatographic peaks of unknowns to their corresponding retention times is considered as a random process. For example, polymer mixtures having similarities in terms of molar mass distribution (MMD) but differ in their molecular architectural distribution (MAD) often cannot be resolved by two-dimensional chromatography. Thus, additional identification often provided by spectroscopic[9] and spectrometric techniques is required, considering these techniques[25-27] as an extension of detection into the third-dimension.[22] The following chapters will summarize developments in the scope of chromatographic hyphenation techniques and their applications in studying complex polymers revealing their specific property distributions.

1.1 MOLECULAR ARCHITECTURES OF SYNTHETIC POLYMERS

Synthetic polymers exhibit complex property distributions and are defined to be "heterogeneous" in view of chemical and structural parameters[28-31], as illustrated in **Figure 1**. Even within the polymer chains, the most preponderant heterogeneity is the one with respect to the molar mass distribution (MMD).[32, 16] The final properties of polymers depend not only on the molar mass distribution (MMD), but also on additional key parameters as *e.g.* chain configuration (*cis-trans* isomerism), conformation or inter- and intramolecular supramolecular interactions. Furthermore, the polymer topologies, as shown in **Figure 1A**, including linear-, branch-, star-, graft- and cyclic polymers, which very often follow a quite difficult scheme of synthesis, can drastically affect the overall properties of polymeric materials.[33]

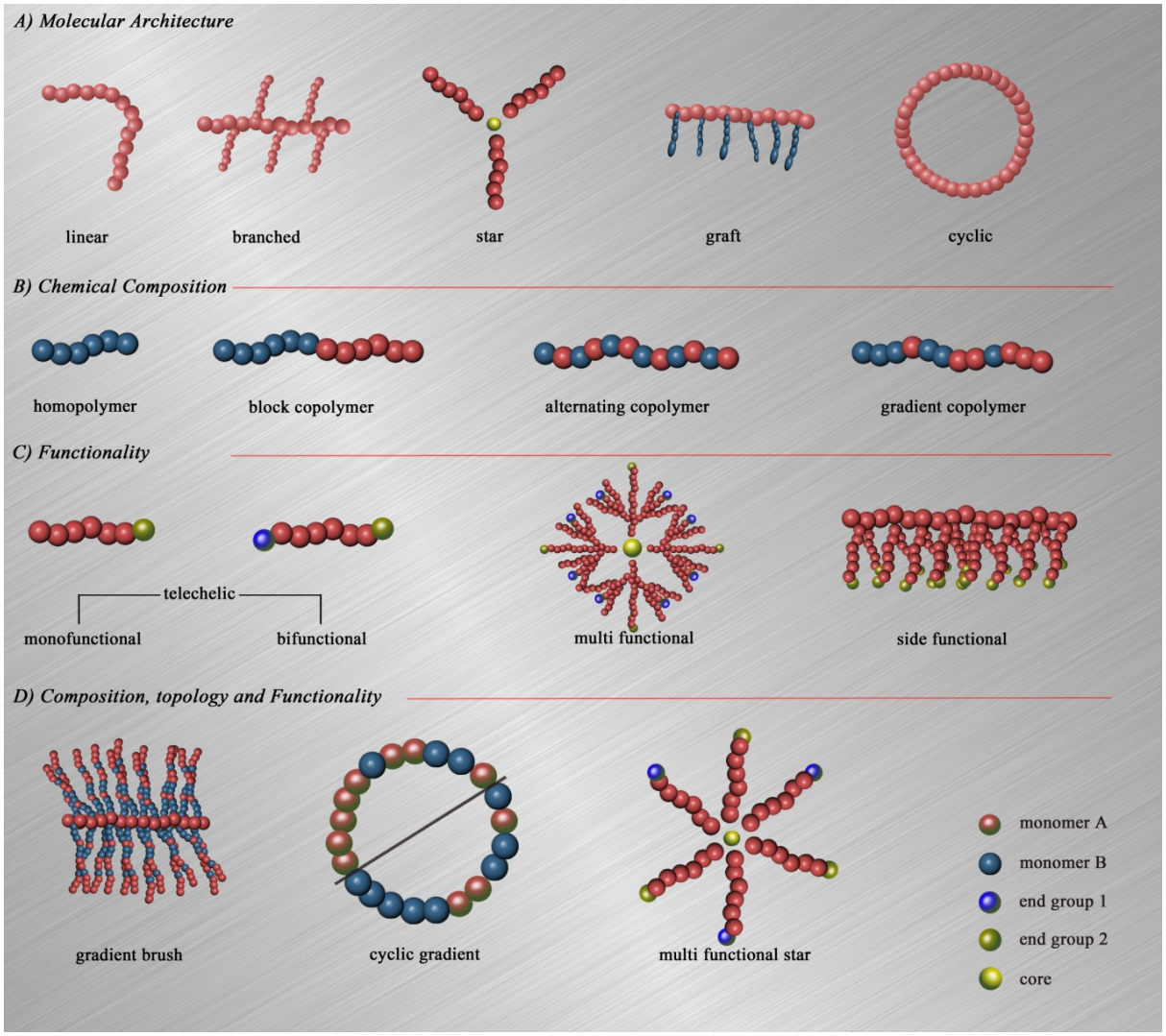


Figure 1. Molecular heterogeneity of synthetic polymers demonstrated on the basis of A) molecular architecture of polymers, B) chemical composition of copolymers, C) end group distribution within the polymeric chains and D) advanced polymeric topologies combining functionality, chemical composition and molecular architecture.

Tailor-made polymers excepting biomacromolecules display a stochastic distribution of chain lengths caused by the polymerization processes.[34-36] These chains can differ in the monomer composition and linkage, prevailing some constitutional features that brings up variation in terms of the structural composition, which lead to the so-called chemical composition distribution (CCD).[13, 37] The monomer distribution within the polymer backbone (monomer A and B), as illustrated in **Figure 1B**, draw out the structural configuration of copolymers, *e.g.* alternating (periodic),[38] random, gradient and block copolymers.[39, 40] Nevertheless, it is significant to mention that the molecular size in solution is influenced by the branching of the polymer (molecular architecture distribution, MAD) and to a less extent by the chemical composition via the affinity of polymers towards particular solvents.[41]

Modification of the polymer backbone by introducing specific groups as side chains or chain ends give rise to a functionality type distribution (FTD).[42, 43] Such polymers are called telechelic polymers and they can be classified into mono-, di-, tri-, or multifunctional (polytelechelic) polymers (see **Figure 1C**). The molecular functionality (f) can be described quantitatively as the ratio of the total number of functional groups attached covalently to the chain ends to the total number of chains within the molecules.

$$f = \frac{Number\ of\ functional\ groups}{Number\ of\ polymer\ chains}$$

This approach can be used to circumscribes the efficiency of end group modification if the number of end groups in a given molar mass of a polymer is known.[44, 45] In the past decades, rapid expansion of polymeric material applications demands the design of complex polymeric architectures, which further need to be intensively studied and proved by novel analytical techniques. These polymers show chain organization that represent an advanced level of topologies.[46-48] The topological forms of such polymers including cyclic gradient copolymers[49] or star-shaped multi functional polymers[50] (**Figure 1D**) are the result of consecutive synthetic steps.

1.2 ONE-DIMENSIONAL CHROMATOGRAPHIC SEPARATION MODES OF SYNTHETIC POLYMERS

Chromatography is an analytical technique, which describes the differences in rates of analytes migration in a liquid or gas mobile phase through a porous medium (stationary phase.[51] The molecules undergo a certain interaction depending on the type of mobile phase and stationary phase. The latter can be chosen to determine the separation mechanism of macromolecules based on molecular exclusion, ion exchange, affinity or adsorption interaction,[52] as summarized in **Figure 2**.

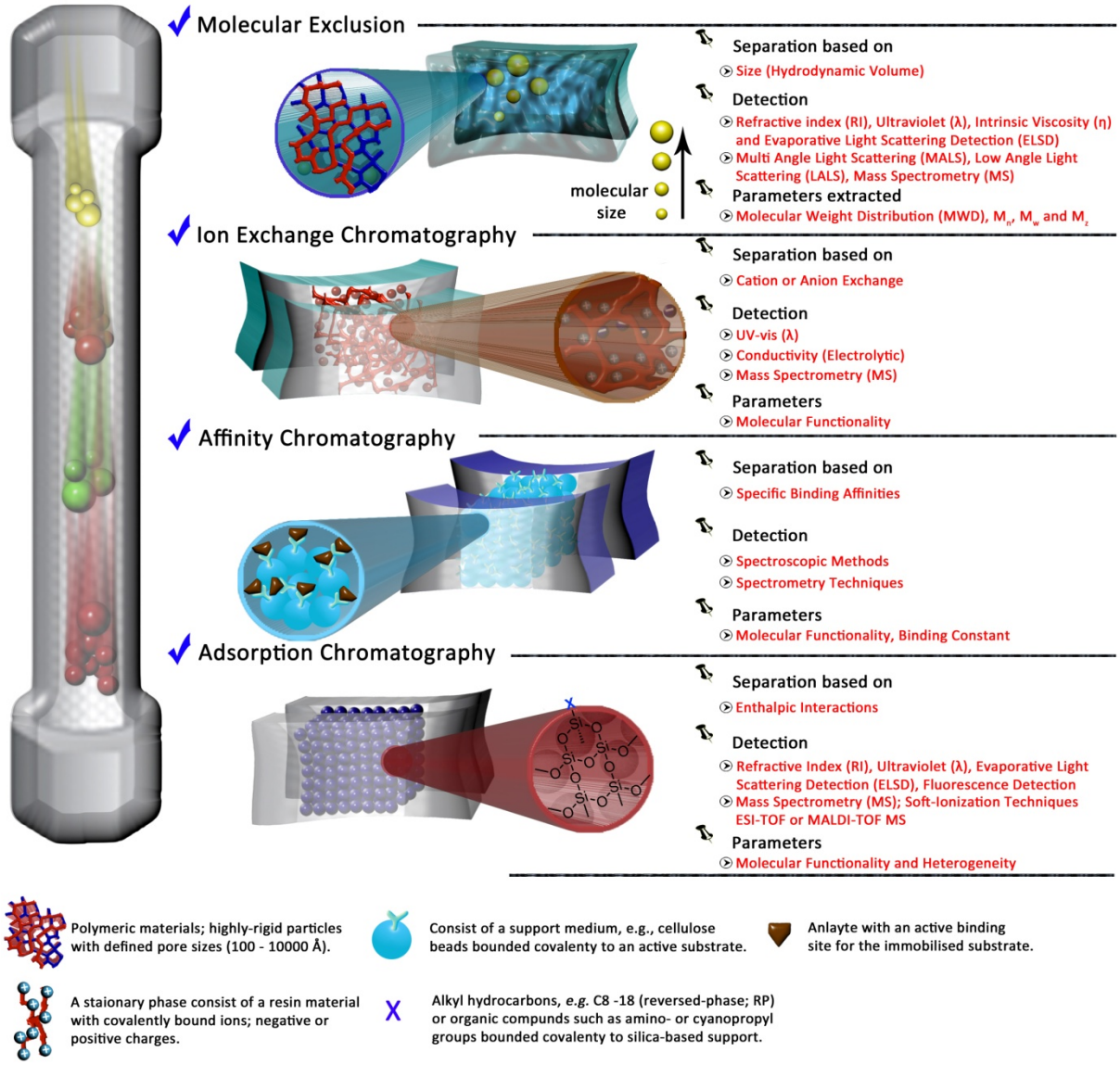
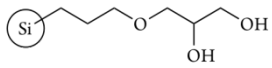
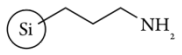
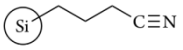
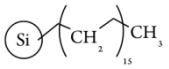
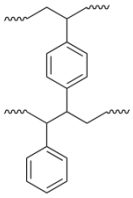


Figure 2. Chromatographic separation modes classified by the supporting medium (stationary phase), along possible methods of detection and parameter outcome.

Two major factors influence the separation mechanism (interaction processes). In adsorption chromatography, the stationary phase based on surface modified silica (*e.g.* modified with alkyl hydrocarbon chains (C8 to C18) termed as reversed phase (*RP*)) support hydrophobic interactions and consequently, enables the adsorption of hydrophobic substances. In contrast to that using silica with active silanol functional groups, the medium becomes strongly hydrophilic and hence interacts with polar substances (termed normal phase; *NP*). To enhance a selective separation process, silanol groups can be modified with organic moieties such as amino- (*-NH_2*) or cyanopropylsilyl- groups (*-CN*), which in turn changes the polarity of the supporting medium and allows selective interactions. In **Table 1**, examples of commercial stationary phases are given.

In exclusion chromatography, the column packing plays an important role in separating synthetic macromolecules based on their sizes using organic solvents or an aqueous medium as mobile phase.[53, 54] Waters Associates (*Milford, Massachusetts*) have introduced the first micro-particles of rigid highly cross-linked polystyrene particles (10 µm), which resulted in high-resolution and selectivity towards organic substances, maintaining a high pressure (up to 20 MPa) and holding more sample load (up to 1000 µL).[55] Such conditions allow the separation of polymers on a preparative scale. Silica[56, 57] and alumina[58] were investigated to cover a wide range of polymers with lower adsorption effects on particle walls.

Table 1. List of selected commercial silica- and polymer-based HPLC columns.

Commercial brand	Chemistry		Structure	Specifications		
	Type	Polarity		Pore size [Å]	Particle size [µm]	pH
Nucleosil-OH	NP -Diol	polar		100	3.5, 5, 10	2-8
Nucleosil-NH_2	NP - NH_2	polar		100	3, 5, 10	2-8
Nucleosil-CN	NP - CN	polar		300, 500	3, 5, 10	2-8
Silica gel YMC	RP - C18	non polar		120	5	2-10
SDV[a)]	SEC	non polar		100 - 10^3	10	--[c)]
PFG	SEC	non polar	Modified silica[b)]	100 - 10^3	5, 7	--[c)]

a) High-speed column.
b) Chemical structure of the modified silica is not given.
c) Effluent *pH* range is not provided.

The second major factor is the mobile phase, where the solvent's polarity-strength (*relative polarity*) controls the interaction behavior of the analyte with the stationary phase, *i.e.* the mobile phase molecules compete with the analyte molecules by displacing them away from the active medium sites and eventually, allowing them to elute out faster from the column.[59-61] Extensive studies showed that optimizing the mobile phase enhances the selectivity of separation, which can be reached by balancing the mobile phase composition either in an isocratic elution, where the composition of the mobile-phase remains constant throughout the chromatographic run, or in a gradient mode.[62, 63] The latter involves two or more different solvents, which differ significantly in the total relative polarity. During the elution, the ratio of solvents is varied either in a programmed way continuously or in a series of steps. As a result, the separation efficiency can be enhanced.[64-66]

Polymers have a characteristic behavior in liquid chromatography, once the elution components are changed, they interact diversely. The retention behavior of polymer molecules depends primarily on both, the mobile- and the stationary phase system. In general, the elution time or retention time of molecules is called peak elution volume and is a consequence of a selective interaction with the mobile phase and the stationary phase, [48, 67, 68] as described by eq. 1,

$$V_e = V_i + V_p K_d \qquad (1)$$

where, V_e is the elution volume of the analyte, V_i is the interstitial volume of the column, V_p is the volume of pore region in the column and the distribution coefficient K_d is the ratio of the analyte concentrations in the stationary phase to that in the mobile phase. Based on Martin's rule,[69] the retention behavior of polymer molecules is related to a dimensionless retention factor k'.[70] This factor increases exponentially with the increase of the repeating unit of a homologous polymer. Based on the factor $k,'$ the distribution coefficient K_d, can be obtained experimentally so that a comparison between chromatographic separation modes is feasible, as demonstrated in eq. 2

$$k' = \frac{V_e - V_0}{V_0} = \left(K_d - 1\right)\frac{V_p}{V_0} \qquad (2)$$

where, V_0 is the void volume of the system, which refers to the volume between the injector and the detector. It can be used to predict the elution behavior of the polymer molecules in an already defined system.[71] The variation in *Gibbs free energy* (ΔG) among polymer molecules migrating through the interstitial and pore volume is also related to the distribution coefficient K_d. According to the *Van t' Hoff plot,*[72] the enthalpy and entropy contributions in a chromatographic system can be determined by the following equation (eq. 3),[70]

$$\ln K_d = - \frac{\Delta H}{RT} - \frac{\Delta S}{R} \qquad (3)$$

In diluted conditions, a large entropy contribution is gained by the diffusion of polymer chains and mobile phase molecules within the stationary phase. Yet, unlike freely diffusing small molecules, the chain mobility of a macromolecule is significantly lower.[13] As polymer molecules enter the pores, structural chain confinements take place losing parts of their conformational entropy, whereas molecules which cannot penetrate the pore dimensions keep their conformational entropy (ΔS) and stop their pore permeation.[73, 74] Small molecules such as solvent molecules, which access the entire pore volume, demonstrate a high degree of freedom compared to macromolecules, allowing them to

stay longer and thus maximize their interactions with the pore walls, which results in a gain of enthalpy (ΔH).[44, 75-77]

To achieve information about the molar mass distribution, one has to consider the entropy change (ΔS) as the driving force and the operating temperature during the separation mechanism ($T\Delta S >> \Delta H$). Another approach of polymer chain separation according to their size have been investigated by maximizing the enthalpy interaction using an active packing material and varying the temperature through a preprogrammed temperature gradient profile (*temperature gradient interaction chromatography*; TGIC).[78] The first successful example of TGIC was performed using polystyrene standards ranging from 2 - 2890 kg/mol in an isocratic solvent mixture of methylene chloride and acteonitrile (ACN) at a mixing ratio of 57/43 using a revered phase column.[79] In general, TGIC is considered advantageous over the solvent gradient method in terms of base line drifting that may occur in some detection methods such as light scattering, differential refractometer and viscosimetry.[78]

Summed up, information about the functionality and chain architecture of polymers requires different strategies. The separation mechanism of polymeric mixtures exhibiting entropy effects can be utilized only if the chain size is different. Otherwise, mixtures having indistinguishable chain sizes, which differ *e.g.* in their functionality or structural organization, a separation is impossible by applying exclusion methods, as it demands methods that are restrained by enthalpic interactions ($\Delta H >> T\Delta S$).[80] An adequate separation of polymer molecules suggests a compensation of the enthalpy and entropy at a defined temperature ($\Delta H = T\Delta S$), *i.e.* at critical conditions (LCCC). Hence, the use of functional materials as stationary phase in the presence of a good solvent or a solvent mixture with a non-solvent can be used to reach this compensation, demonstrating a molar mass independent elution of the chains.[44, 81, 82]

1.2.1 SIZE EXCLUSION CHROMATOGRAPHY OF SYNTHETIC POLYMERS

Exclusion chromatography is set back in time to 1897, where *David Talbot Day* was able to separate fractions of crude oil by size using bleaching earth.[51] Nevertheless, this phenomenon was not fully understood until the original founding by *Tswett* in 1906.[16] Herewith, the first water soluble macromolecules were investigated using polydextran gels and later commercialized for gel chromatography techniques.[83] *Lea* and *Sehon* prepared synthetic gels by modifying polydextrans to diethylaminoethyl-dextrans, which were used for ion-exchange and further for synthesis of various hydrophilic polymers and gels.[84] *J. C. Moore* in 1964 has taken advanced measures toward the separation of various sizes of synthetic polymers by synthesizing cross-linked polystyrene beads as a rigid medium support (column).[85] Polystyrene (M_n = 13 - 3500 kg/mol) and polypropylene glycol samples were investigated using a mixture of hydrophobic solvents. Since polystyrene interact diversely based on the physical and chemical properties of chains with different columns, *J. C. Moore* introduced a calibration curve supported by experimentally probed permeability factors, which provided information about the molar mass distribution (MMD) of PS and PEG.[85] *Bly* reviewed the results of his work later in 1969.[86] Therefore, universal calibration of nonionic polymers such as polyethylene oxide and polysaccharides can only be valid once a nonionic SEC system is applied.[87] A recent study using pulsed laser polymerization combined with SEC revealed an accurate relation for the determination of the kinetic rate constant, which delivered a precise SEC calibration with narrow standards. Thus, it was stated that the accuracy of *Mark-Houwink parameters* affect the calibration in SEC, *i.e.* the hydrodynamic volume extracted from those narrow standards does not accurately represent the viscosity-MMD curve for masses ≤10 kg/mol.[88]

However, a combination of viscosity and concentration detectors can be applied to transform the values of the hydrodynamic volume to more accurate molar masses. Still, this method suffers from accuracy in terms of polymer concentration and the delay time resulted by interconnecting the detectors either in serial or in parallel modes.[89] Another detection method for the determination of molar masses is light scattering (LS). The molar masses are calculated by comparing the intensity of the scattered light at defined angular dependence (θ) with the incident beam, which precisely provides information about the polymer dimensions by means of the mean square of the radius of gyration.[90] In size exclusion chromatography, concentration sensitive detectors are at least interconnected with light scattering detectors, *e.g.* MALS, RALS or LALS (see **Table 2**), as the latter is considered only sensitive towards high molar masses under diluted conditions. Furthermore, it was reported that polymers with low molar masses would bias if the sample concentration is

insufficient.[91] A large variety of different detection methods allow beside the molar mass distribution to obtain additional information about the composition and functionality in complex polymer samples (**Table 2**).[90]

Table 2. Molecular distribution of polymers and their corresponding methods of separation/detection, reprinted with permission from [92]. Copyright (2004) American Chemical Society.

Macromolecular properties	Molecular distributions	Separation methods [(a)]
Molar mass	MMD	**SEC**, FFF, HDC, TGIC, CEC, SFC
Long-chain branching	MAD	**SEC**-MALS, **SEC**-VISC
Short-chain branching		**SEC**-IR, **SEC**-NMR, TREF
Cross-linking		**SEC**-MALS, SEC-VISC
Architecture		**SEC**-MALS-QELS-VISC, 2D-LC-**SEC**-MS
Tacticity	CCD	**SEC**-NMR, TGIC, LCCC
Chemical composition		GPEC, TGIC
Chemical Heterogeneity	FTD	**SEC**-spectroscopy, **SEC**-spectrometry, 2D-LC-SEC

Many techniques require **(a)** concentration-sensitive detector (*e.g.* a differential refractometer), not included here for simplicity.
*__SEC__: size-exclusion chromatography, **FFF**: field-flow fractionation, **TGIC**: temperature-gradient interaction chromatography, **CEC**: capillary electrokinetic chromatography, **SFC**: supercritical fluid chromatography, **MS**; MALDI-MS: matrix-assisted laser desorption/ionization mass spectrometry, **MALS**: multi-angle light scattering, **VISC**: viscometry, **IR**: infrared spectroscopy, **NMR:** nuclear magnetic resonance spectroscopy, **TREF**: temperature rising elution fractionation, **QELS**: quasi-elastic (dynamic) light scattering, **LCCC**: liquid chromatography under critical conditions, **GPEC**: gradient polymer elution chromatography, **2D-LC**: two-dimensional liquid chromatography.

Recently, a combination of SEC and multiple detection systems (compare **Table 2**) such as light scattering (LS), Viscosity, refractive index (RI) and/or ultraviolet (UV) have been developed to gain more insight on the structural variation of polymers under investigation.[93]

1.2.2 LIQUID ADSORPTION CHROMATOGRAPHY (LAC) OF SYNTHETIC POLYMERS

The behavior of macromolecules on solid surfaces represents the physical boundary of chain dynamics in solution. The interaction of single chains in the vicinity of a surface can be described as a competition between the energy gained by adhesion of chains to the surface and the entropy lost subjected to chain configuration.[94] The energy compensation by this interaction results in multiple configurations of the chain influenced by the number of visits to the surface layers.[71] This mechanism represents a number of structural conformations that have been studied extensively by theoreticians,[95-103] who demonstrated enthalpy-entropy two-dimensional structural configuration models generated by adsorption on surfaces in solution.

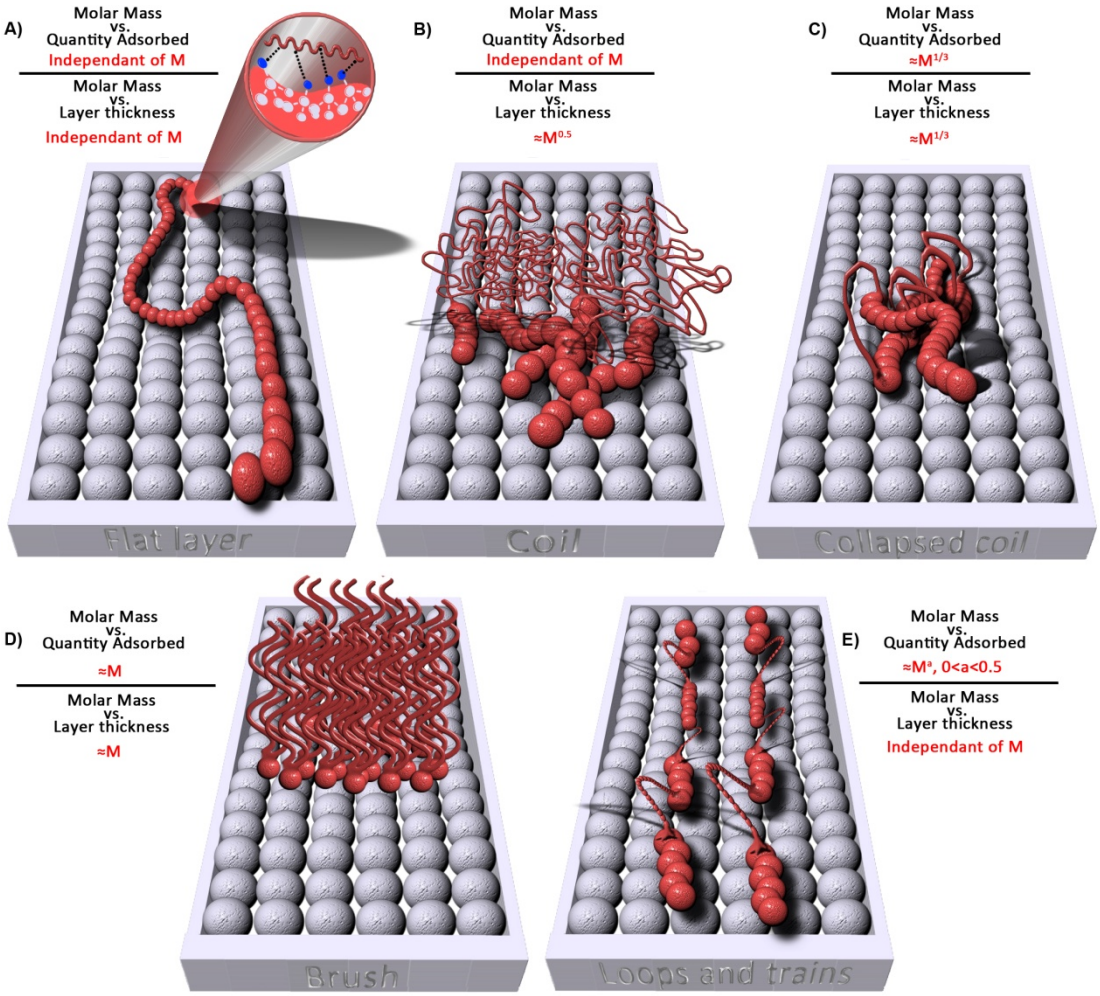


Figure 3. Adsorption models of macromolecules on silica medium support as suggested by Ullman in1964.[104] (A) Shows the flat layer, (B) coil, (C) collapsed coil, (D) brush and (E) loops and trains model of adsorbed polymer chains.

These structures that are often in a non-equilibrium state with the substrate, persist on the surface due to the high free energies of activation. The adsorption takes place in the presence of a set of adjacent molecules (blocks) being adsorbed on the basis of adsorb-capable functional groups. But molecules that form a thermodynamically stable coil conformation are the least adsorbed on the substrate (see **Figure 3B**). Once the equilibrium will be changed (*elution components*), the stable coil would transform into various conformations. As illustrated in **Figure 3A**, the maximum entropy loss of the chains due to the maximum enthalpy gain would stretch the coil into a flat layer, which was first suggested by Ullman et. al.,[104] and later confirmed by G. Glöckner[105, 106] and H. Pasch in 2005.[107] Moreover, the flat layer model would preferably take place only if a sufficient space is available on the substrate.[101]

Lowering the interaction between the polymer molecules and the surface can be achieved by replacing the adsorbed chains with a proper solvent, *i.e.* by manipulating both the eluotropic strength of the solvent with respect to the stationary phase. These models (**Figure 3**) and their formation are intercorrelated with other parameters such as temperature. Small molecules based on negative adsorption enthalpy show less adsorption at higher temperatures,[108] whereas macromolecules showed the same effect at much lower temperatures.[109, 110] Entropy effect at higher temperatures plays a crucial role in shaping the chains into different conformations, which in turn change the adsorption behavior on the substrate entirely. This behavior is further related to the polarity strength (P) of the solvent (mobile phase) and the surface porosity of the substrate.[65] The solvent quality completely determines the adsorption behavior of the chains on the substrate (stationary phase). For example, poor solvents generally show better adsorption than thermodynamically good solvents.[111] Polar solvents tend to remove all adsorption layers on polar/non-polar substrates and polar solvents with a high dielectric constant, showed the same effect however, by shielding the chains from adhering the substrate.[112, 113]

Adsorption methods are usually applied to gain information on the chemical composition (CCD) of polymers, basically on homo-, co- and telechelic polymers. As the separation mechanism is mainly based on the chain size,[114] differences in the chemical structure *e.g.* end groups and in architecture may overlap. In order to achieve a successful separation by end groups or other small structural differences one has to choose conditions, which allow a molar mass independent chromatographic elution (LCCC).[115]

1.2.3 LIQUID CHROMATOGRAPHY UNDER CRITICAL CONDITIONS (LCCC)

Polymer chain separation is strongly influenced by the components used in liquid chromatography (*i.e.* composition of mobile phase, type of stationary phase and column temperature). In particular, the chain retention behavior in a chromatographic column depends on the adjustment of all the above-mentioned parameters, which has to be probed individually and carefully for each polymer sample.[116] In reversed phase substrates (usually silanol modified with *n*-alkyl-chains),[117] only non-polar chains or non-polar functional moieties adsorb on the substrate via formation of electrostatic attraction, hydrogen bonding or *van der Waals* forces.[60, 61, 118]

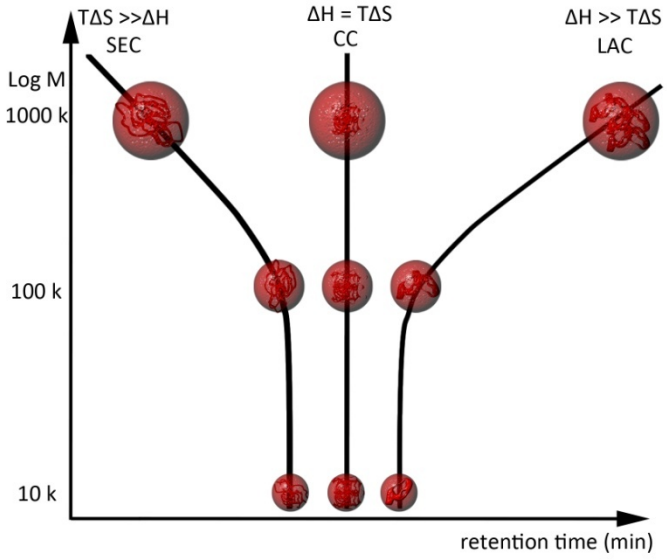
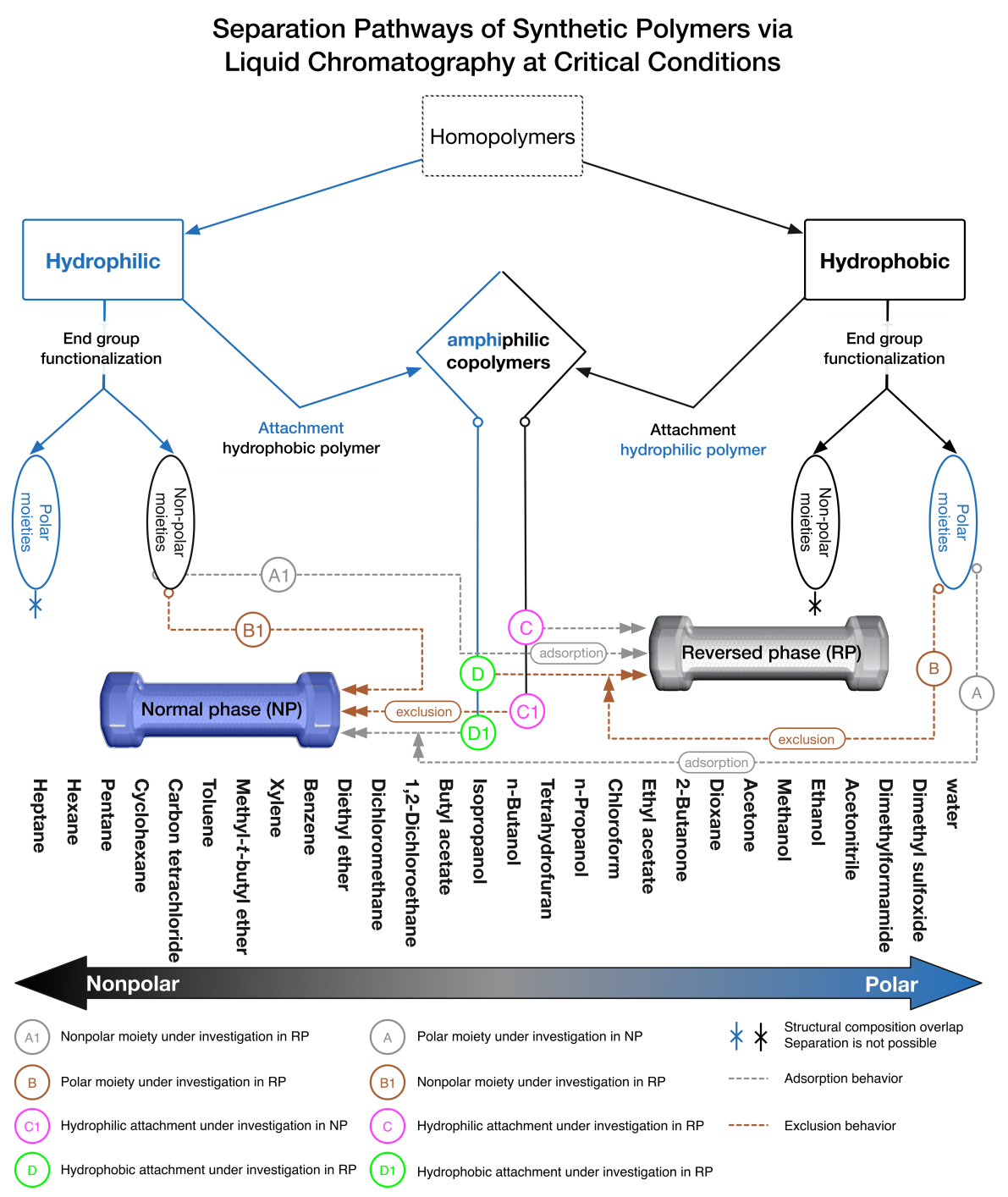


Figure 4. Retention behavior of macromolecules in size-exclusion mode(SEC), under critical conditions (CC) and adsorption interaction mode, as a function of the molar mass.

Manipulating these effects can lead to a special mode of separation that force long and short chains to coelute at a critical point, where enthalpy come into a balance with the entropy. Consequently, a molar mass independent elution of polymer chains is observed, as illustrated in **Figure 4** ($\Delta H = T\Delta S$). These critical conditions (CC) were experimentally obtained and probed for a variety of functional polymers[115] and later on for copolymers.[119, 120] The transition between adsorption and exclusion was first demonstrated for mono- and bifunctional polybutadiene oligomers, finding critical conditions of the homologous polymer in different solvents such as heptane, hexane and chloroform.[115] Thereafter, CC of adipic acid-dietheylene glycol copolymer was identified using a solvent mixture of methyl ether ketone/hexane, (92/8, v/v), which showed an improved chromatographic separation between linear and cyclic glycol copolymers.[121] The chromatographic invisibility of chains regardless their size made a plenitude of achievements in separating cycles from linear polymers, copolymers from their homopolymers and functionalized polymers from the non-functionalized ones in a quantitative regime.[122-124]



Scheme 1. Separation strategies of functionality type distribution (FTD) and chemical composition distribution (CCD) of post-modified polymers and copolymers, respectively.

The outstanding results following the critical conditions in separating various macromolecules made a great progress up-to-date.[125] Scientists utilized the discovery of critical conditions for many different synthetic polymers (*e.g.* PS, PMMA, PEO) using an isocratic elution mode and commercially available stationary phases, as summarized elsewhere.[126]

By screening the experimental progress, a schematic strategy based on the hydrophilic or hydrophobic character of polymers can be found, which provides guidance in finding the proper conditions of a molar mass independent elution of chains, as illustrated in **Scheme 1**. For example, a hydrophobic polymer modified with polar end groups; mono- or bifunctional moieties (Scheme 1, route *A* or *B*) can be identified by using either a reversed or a normal phase column. The latter decides the separation effect by the end group functionality. Route *A* in Scheme 1 (adsorption effect) provides additional information on the structural distribution of chains in a modified polymer sample, examples are mentioned elsewhere.[108, 115, 127-131] Route *B* (exclusion effect) determines the efficiency of end group functionalization and thus the purity of the final polymer.[12, 26, 81, 88, 105, 132-140] Moreover, symmetrical or nonsymmetrical bifunctional end groups (polar character) of modified polymers were also observed to exhibit a SEC retention behavior on a reversed phase stationary column (**Scheme 1**, route *B1*).[141-144] These conditions are related to the nature and porosity of the stationary phase and the composition of the mobile phase, which remains constant (isocratic experiment) at a defined temperature.[145, 146]

The determination of the critical conditions is usually a time-consuming process and is considered very sensitive towards the chemical nature and composition of polymers.[116, 126] It is also evidenced that small changes in temperature and sometimes in column pressure influence significantly the interaction behavior of chains during the elution.[78, 147-150]

1.3 TWO-DIMENSIONAL CHROMATOGRAPHIC SEPARATION MODES OF SYNTHETIC POLYMERS

The separation of macromolecules and other complex materials, including more than one property distribution cannot be addressed by one-dimensional chromatographic methods.[151] Two-dimensional experiments can overcome the limits of a 1D- chromatographic separation, which was demonstrated for the first time by *Markham* and *Smith* by spotting purines, pyrimidines and their ribosides on paper chromatograms. By using different solvents (employing their R_f values), spots were then printed on a photographic paper.[152, 153] 2D-planar chromatography were further employed to investigate lipid separation[154] and poly(adenosine diphosphate ribose).[155] This is considered as the simplest form of 2D-chromatography, where analytes are spotted on a planar support and eluted with a defined mobile phase. The medium support is subsequently rotated by 90° and analytes are again eluted in the second direction by a different mobile phase system. Consequently, choosing a mobile phase with a distinctive strengths and selectivity controls the adsorption, exclusion or even another behavior, resulting in a separation of molecules in orthogonal directions instead of one.[156]

Thereupon, extraordinary achievements have been established in liquid chromatography and column technology to enable the identification of mixtures in solution.[58, 157] Albumin solutions were first characterized "*quality controlled*" by the so-called SE-HPLC (Size-Exclusion High-Performance Liquid Chromatography) using a combination of molecular exclusion and adsorption.[158] Complex polymers require an accurate molecular characterization combining one-dimensional techniques, which separates upon functionality with molecular size into a two-dimensional chromatographic system.[159] Therefore, it is beneficial to couple chromatographic techniques to determine distributions of various properties in order to better understand the structure- property relationship of polymers.

Experimental developments were further extended to use switching valves having eight or ten ports, which collect fractions from the first dimension into a transfer loop while the content of a second loop being injected and analyzed by the second dimension[160], as shown in **Figure 5**.

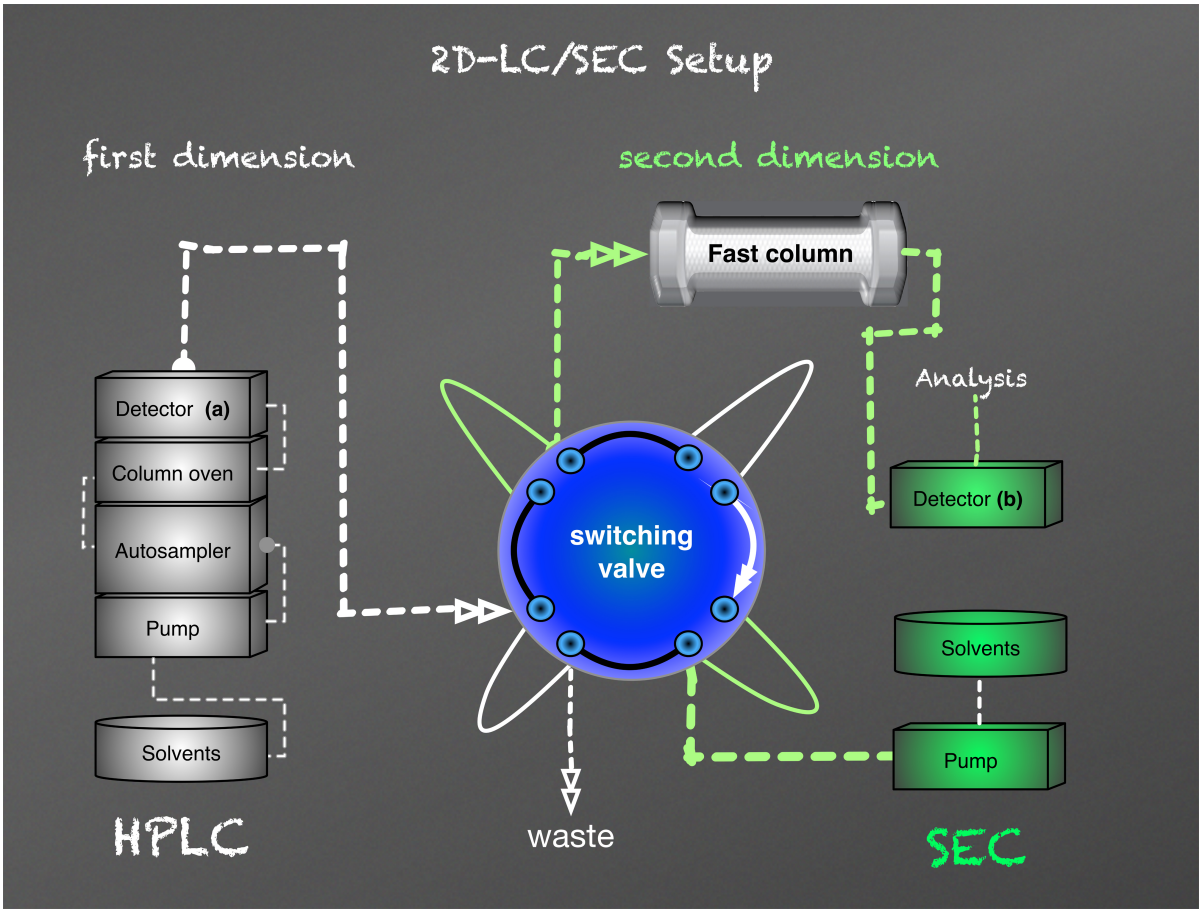


Figure 5. Schematic overview of a fully automated two-dimensional chromatography setup utilizing adsorption or critical conditions as the first dimension and size-exclusion as second dimension.

The selectivity of the dimensions are based on the differences between specific and nonspecific interactions such as polarity and architecture, or charge (ionic interactions).[161] Some important methods including critical conditions of polymers in predefined solvents (LCCC), a gradient elution profile (GPEC) mode or temperature gradient interaction chromatography mode (TGIC) were experimentally probed.[162-165] A summary of important polymer classes investigated via a combination of these modes versus the exclusion mode in 2D-chromatographic systems is demonstrated in **Table 3**.

In two-dimensional systems, LCCC is considered as a chromatographic separation technique, which conveys homogeneous fractions with respect to the chemical composition of molar mass independent polymer chains. The separated fractions can be analyzed by SEC that provides after the first dimension an information about their molar mass distribution (MMD).[17] Such combination is considered as "orthogonal". For example, aliphatic polyesters with respect to end group functionality and molar mass distribution were analyzed by a comparable method.[166-168] The critical point of adsorption of the polyester chains was found to be at the composition of acetone to hexane of 51 to 49 (v/v) utilizing a silica gel column as stationary phase. By separating these fractions and

transferring them to SEC (2nd dimension), the MMD of the before separated polyester chains (FTD) was additionally identified. However, the analysis of complex polymers by the second dimension is affected by the peak capacity, which is governed by an orthogonal chromatographic system that give rise to a statistically independent retention volumes, based on both axes.[169]

Table 3. Summary of two-dimensional chromatographic separation conditions of common polymers.

Entry	Polymer	1st dimension (HPLC)						2nd dimension (SEC)
		mobile phase		stationary phase				
		Composition	C[a)]	ID[b)]	S_P[c)] (mm)	V_P[d)] (Å)	D[e)] (cm x cm)	Stationary phase
1	PEO[170, 171]	Methanol/H_2O 86/14 (v/v)	LCCC	Synergi MAX-RP	4	80	25 x 0.46	Synergi Fusion RP: silica-based dodecyl phase ; 80 Å (25x4.6 mm I.D.), S_P[c)] 4 µm; (Phenomenex)
		ACN / water 43 /57 (v/v)	LCCC SFC	Nucleosil-C18	5	100	25 x 0.40	*Ultrastyragel* 1000 Å, S_P[c)] 5 µm, (300 x 8 mm I.D.), 2 x 500 Å, S_P[c)] 5 µm, (300 x 8 mm I.D.) and *Waters* 2 x 100 Å, S_P[c)] 5 µm, (300 x 8 mm I.D.)
2	PMMA[172]	THF/n-hexane 82/18 (v/v)	LCCC	Nucleosil-NP + Nucleosil-NP	5	100 + 300	15 x 0.40 + 25 x 0.40	Two linear *SDV* (*PSS*, Mainz), S_P[c)] 5 µm (300 × 8 mm I.D.) as well as a guard column (50 × 8 mm I.D.)
3	PBA[173]	THF/Cyclohexane 15.5/84.5(v/v)	LCCC	Silica gel + Silica gel	10	300 + 1000	25 x 0.40	*PL-gel* Mixed D, S_P[c)] 5 µm, (300 mm × 7.5 mm, I.D.)
4	PtBMA[174, 175]	ACN/THF	G[f)]	Nucleosil-C18	5	100	25 x 0.40	Linear *SDV* (*PSS*, Mainz), S_P[c)] 5 µm (300 × 8 mm I.D.)
			LAC	Zorbax-C8	5	100	15 x 0.46	
		THF/ACN, 49.5/50.5 (w/w)	LAC	Nucleosil-C18 + Nucleosil-C18	5 + 7	100 + 1000	25x0.40 + 25x0.40	Two linear *SDV* (*PSS*, Mainz), S_P[c)] 5 µm (300 × 8 mm, I.D.), guard column (50 × 8 mm, I.D.)
5	PEMA[176]	THF/Cyclohexane 64/36 (w/w)	LCCAP	Develosil-NH_2	5	120	25x0.80	*PL-gel*, Mixed D, S_P[c)] 5 µm, (300 × 7.5 mm, I.D.)
6	PS[177]	n-hexane/THF 55/45 (v/v)	LCCC	Nucleosil-Si 300	5	300	25x0.46	Linear M SDV (*PSS*, Mainz), S_P[c)] 5 µm, (50×20 mm I.D.)
7	PB[178]	n-heptane/ dioxane 97.3/2.7 (v/v)	LCCC LAC	Nucleosil-NH_2	7	1000	25x0.40	Two Styragel (Waters), S_P[c)] 5 µm, (300 × 7.8 mm, I.D.)
8	PI[179]	MEK/cyclohexane 91.5/8.5 (v/v)	LCCC	Nucleosil-C18	5	300	25x0.40	Linear SDV 10000 Å (*PSS*, Mainz), S_P[c)] 5 µm, (50 × 20 mm I.D.)
9	PIB[26]	MTBE/MeOH 85.34/14.66 (v/v)	LCCC	Atlantis-C18	5	100	25x0.40	PSS *PFG* high-speed (PSS Mainz), S_P[c)] 5 µm (50 × 20 mm I.D.)
10	pCL[25]	DCM/THF 92/8 (v/v)	LCCC	Nucleosil-NH_2	5	100	25x0.40	Linear SDV 1000 Å (*PSS*, Mainz), S_P[c)] 5 µm, (50 × 20 mm I.D.)
11	Polyacetals[180]	HFIP/water 50/50 (v/v)	GPEC	HyPurity Aquastar C18	5	100	25x0.46	HFIP-gel column with 0.05 wt.-% KTFA at 40 °C.
12	Polyesters[181]	Acetone/hexane 51/49 (v/v)	LCCC	Nucleosil-Si	5	50 100 300 500	25x0.46	PL-gel column, 50, 1000 Å S_P[c)] 5 µm, (300 mm × 8 mm, I.D.)

a) C, chromatographic separation mode; **b)** ID, column identity "commercial brand"; **c)** S_P, particle size in µm; **d)** V_P, pore volume in Å; **e)** column dimensions in cm; **f)** G, gradient elution mode.

The degree of orthogonality (O) in a 2D system is obtained by the normalized area covered by the eluting peaks in a separation plane divided into a discrete number of space elements (bins), which may contain one sample compound for each.[24] Several experimental factors such as plate count, injection volume, injected mass and injection band dilution must be considered when designing a two-dimensional separation system. Thus, it is clear that a low resolution and low injected mass with high dilution of injection cannot be considered for such systems.[182, 183]

New developments in the field of 2D-chromatographic systems were focused on shortening the analysis time. An approach using heart-cutting separations allows only the components of interest to be transferred via a conventional switching valve into the second dimension, where fractions are analyzed on a time basis.[184] In contrast, comprehensive 2D-analysis collects all fractions into the second dimension that consumes more time and effort.[185] Online switching valves using splitless transfer injections were employed on CE, LC and SEC and have proven to be successful, which is demonstrated by the experimental results and the actual number of published papers of 2D separations.[161] The most widely used combination is liquid chromatography based on specific separation modes coupled to SEC. The proper sequence of separation methods is significant for the selectivity and the separation efficiency for the determination of property distributions. Moreover, the best is to apply the method with the highest selectivity for one property as first dimension, ensuring the highest purity of eluting fractions, which are transferred into the subsequent separation.[186]

Gradient elution liquid chromatography is usually used as first dimension in a 2D separation set up.[165, 187-190] This technique has been applied to successfully separate random copolymers, block copolymer, as well as polymer blends.[187, 191-196]

1.4 MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY OF SYNTHETIC POLYMERS

The identification of structural and chemical heterogeneities in polymeric analytes is a difficult task for one- or two-dimensional liquid chromatography. The peak assignment is usually done by comparing the retention times of unknown samples with those of known compounds.[197] A qualitatively analysis of a multicomponent sample by one- or two-dimensional liquid chromatography using only the retention time information is very imprecise and a clear disadvantage in the sample handling.[198, 199]

Table 4. Hyphenated multidimensional characterization techniques of some selected polymers, copolymers.

Entry	Sample	Hyphenated Techniques/Multidimensional Characterization			Outcome
		Chromatography	Spectroscopy	Spectrometry	
1	PI-*b*-PMMA[200]	LCCC × SEC	^{1}H NMR, RI	N/A	• Investigation of the microstructure distribution of the PMMA blocks • Investigation of isomeric unit distribution of PI
2	PS[201]	SEC × SEC	UV, RI	MALDI-TOF MS	• Study of band broadening in two-dimensional SEC demonstrated by chromatographic dispersion
3	PS star polymers[202]	RP-TGIC × SEC 2D-LC	LS, RI, UV, VISC	N/A	• Branching analysis SEC/TD method associated with "local polydispersity" • Co-elution of polymer species of the same size, but polydisperse in both chain architecture and MW
4	PEO-*b*-PIB-*b*-PEO[26]	LCCC × SEC	PDA × ELSD	MALDI-TOF MS	• End group modification • "Click"-efficiency in construction of tri-BCPs • Chemical composition studies
5	Styrene-methylmethacrylate-butylacrylate-butadiene[203]	LC × SEC	FTIR, DVISC	N/A	• "Copolymer concentration and composition along an absolute molar mass axis
6	pCL[25]	LCCC × SEC	PDA × ELSD	MALDI-TOF MS ESI-TOF MS	• "Click"-efficiency of end group modification of hydroxyl-, alkyne- and thymine-functionalized pCLs • Calibration of 2D-LCCC-SEC setup via MALDI-TOF MS
7	Poly(bisphenolA) carbonate [204]	LCCC × SEC	UV × FTIR	MALDI-TOF MS	• Hydrolytic degradation of poly-(bisphenol A) carbonate samples identified by FTIR or MALDI-TOF MS as a third dimension
8	PVP-*b*-PVAc[205]	LC, gradient LC	NMR, LS, UV, FTIR	MALDI-TOF MS	• Elucidation of RAFT mechanism of PVP/PVAc BCPs • Distinguish between main products and by-products of RAFT synthsis via LC-FTIR and LC-MALDI-TOF MS
9	Ethylene/Propylene copolymers(EPC)[206]	SEC, TREF	^{13}C-NMR, FTIR, RI, TDA	N/A	• Determination of isotacticity of EPC • Chemical composition distribution

However, the collection of chromatographic fractions after the second dimension and subjecting them further to additional analytical techniques *e.g.* infrared spectroscopy or mass spectrometry was shown to be very effective in identifying chemical heterogeneities within polymeric samples.[207-213] In **table 4**, a summary of recently published multidimensional characterization techniques is given for some selected polymers and copolymers.

Problems in a multidimensional setup can arise when extensive peak overlap occurs. Especially, in complex mixtures of polymers and only through MS, the exploitation of structure dimension becomes necessary.[25, 26] Recently, we were able to develop a multidimensional chromatographic method coupled to soft ionization techniques to unveil property heterogeneities in triblock copolymers such as the molar mass, chemical composition and end group functionality.[26] It is assumed that a combination of multidimensional chromatographic methods along with spectrometric techniques can be involved to identify compounds that cannot be addressed by one-or two-dimensional chromatography alone.

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OBJECTIVES 2

This thesis investigates the coupling of chromatographic methods to soft-ionization mass spectrometric techniques such as MALDI-TOF and ESI-TOF MS to develop an efficient method identifying complex oligomeric and polymeric mixtures. Herein, the first aim concerns a fast and selective gradient method using HPLC, which separates amino resin oligomers bearing different functional moieties based on their polarity. Liquid adsorption chromatography (LAC) coupled online with a diode array detector (DAD) and an ESI-TOF MS will be used to reveal the chemical structure of the modified oligomers and their degree of functionalization.

In contrast, polymeric mixtures demonstrate multiple or complex molecular distributions compared to oligomeric mixtures, which make it tedious to clearly identify their chemical composition, purity and their structural arrangement by one-dimensional chromatographic separation. To overcome the limitations of such a separation method in terms of complex molecular heterogeneities in polymer samples, multidimensional chromatographic methods coupled online/offline to mass spectrometric techniques will be developed.

Therefore, different model systems based on end group functionalized polymers (*e.g.* poly(ε-caprolactone)s (pCLs), polyisobutylenes (PIBs) as well as di- and triblock copolymers composed of polyisobutylene and poly(ethylene oxide) (PEO)) will be investigated. Thus, telechelic α,ω-poly(ε-caprolactone) samples varying in their molecular weight and end group structure will be analyzed by a two-dimensional liquid chromatographic setup coupled in parallel, offline to MALDI-TOF MS via a sample transfer module (TM sprayer) and online to ESI-TOF MS. For the first dimension, pCL homopolymers will be probed on different stationary phases and effluent mixtures, seeking the critical condition of the polymers, where a molecular-weight independent elution is resulted. This allows further an efficient and selective separation of the telechelic pCLs based only on their functionality (*i.e.* polarity). Subsequently, the second dimension running at a synchronized flow rate with the first dimension would then separate the collected fractions to their respective molecular weights.

As a result of studying pCL homopolymers by such a newly-developed 2D-LC/GPC-MS method, this model system will be used to investigate further homo- and block copolymers from end group functionalized PIBs and PEOs revealing their specific block composition, purity and structural symmetry.

RESULTS AND DISCUSSION 3

The **results and discussion part** of the present thesis is based on the published articles listed below taken with permission of American Chemical Society (ACS) and John Wiley & Sons. The first chapter **3.1** demonstrates a simple coupling technique that describes complete chromatographic separation and spectrometric analysis of functional melamine-formaldehyde oligomers. However, due to the architectural complexity of macromolecules that can be originated from multiple synthetic steps, two-dimensional liquid chromatography assisted with soft ionization techniques were enabled to determine various molecular properties of complex polymeric mixtures such as their molecular weight distribution (MWD), functionality type distribution (FTD) and molecular architectures. This will be presented and described in chapter **3.2** and **3.3** utilizing a self-developed 2D-LC/GPC coupled to ESI- or MALDI-TOF MS technique to confirm the complex heterogeneities within different homo- and block copolymers.

Chapter 3.1

Azide/alkyne-"click"-reactions on amino resin materials: An LC-ESI-TOF analysis

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Chapter 3.2

Multidimensional Characterization of α,ω-Telechelic Poly(ε-caprolactone)s via Online Coupling of 2D Chromatographic Methods (LC/SEC) and ESI-TOF/MALDI-TOF-MS

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Chapter 3.3

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3.1 Azide/Alkyne-‘‘Click’’-Reactions on Amino Resin Materials: An LC-ESI-TOF Analysis

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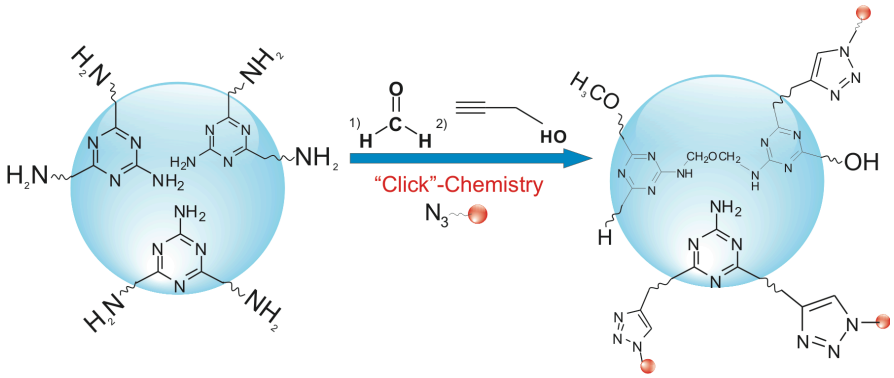
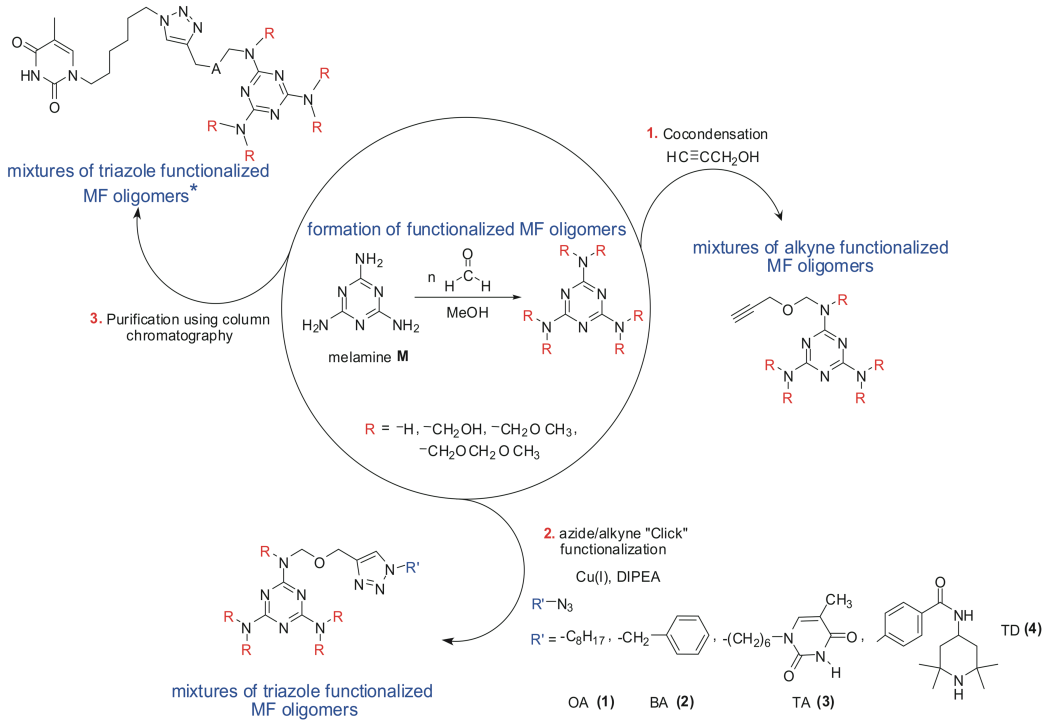


ABSTRACT: This article describes the reaction of amino resins with functional molecules using the azide/alkyne-“click”-reaction, opening a simple chemical modification of amino resins under aqueous conditions. Alkyne-modified melamine-formaldehyde resins are prepared via a direct co-condensation approach using propargylic alcohol (21.6–86.3 mmol) as additive. Subsequently, alkyne-modified mono-, bi-, and trinuclear melamine-species are identified via LC-ESI-TOF methods proving the covalent incorporation of alkyne-moieties in amounts of up to 3.9 mol %. Subsequent modification of the alkyne- modified resins was accomplished by reaction of functional azides (octyl azide (**1**), (azidomethyl) benzene (**2**), 1-(6-azido- hexyl) thymine (**3**), and 4-azido-N-(2,2,6,6-tetramethylpiperidin- 4-yl)benzamide (**4**)) with Cu(I)Br and DIPEA as a base. The formation of triazolyl-modified MF-resins was proven by LC-ESI- TOF methods, indicating the successful covalent modification of the amino resin with the azides **1**–**4**.

INTRODUCTION

The cocondensation of 1,3,5-triamino-triazine (melamine) and formaldehyde yields amino resins, which are widespreadly used in their technical application as impregnation resins, adhesives, or binders within wood-based panels.[1,2] As melamine/formaldehyde resins (MF-resins) contain considerable amounts of water (often up to 50 *wt* %), subsequent chemistry on these resins is limited to reactions taking place under a strictly aqueous environment.[3] Moreover, being precondensates with limited stability,[4–7] chemical modifications are limited to only small changes in chemistry, mostly achievable by large-scale cocondensates (such as urea or phenol) or small-scale additives to improve their UV-, thermal-, and chemical stability. A well-known type of chemical modification is the use of aliphatic alcohols applied in large amounts in mixture with methanol, thus generating modified methylated or butylated melamine resins with adjustable hydrophobicity or hydrophilicity. However, besides these sets of reactions that are based on a direct cocondensation reaction between the pendant amino/hydroxymethyl moieties attached to the amino-triazine moieties, a general concept for the covalent modification of amino resins is yet to be found. In the current approach, we report on the use of the azide/alkyne-“click”-reaction[8–10] to achieve a chemically broad, “modular” and efficient modification of methoxylated MF-resins, able to covalently modify the resin with a broad range of chemical functionalities (Scheme 1). The use of the azide/ alkyne-“click”-reaction has been demonstrated largely on polymeric materials,[11–15] including graft-polymers and polymer networks, thus proving its broad applicability and a high level of completeness in meeting the high standards necessary for completely functionalized polymers. Most of all, the azide/alkyne-“click”-reaction has been demonstrated as an efficient crosslinking reaction on multifunctional amines;[16–18] thus easily envisioning the application of this reaction for the functionalization of MF-based resins. As the azide/alkyne-“click”-reaction is highly efficient in an aqueous environment,[9] the reaction is ideal to permit the covalent attachment of functional moieties onto the resin material. Moreover, as the mechanism of the azide/alkyne-“click”-reaction is based on a 1,3-dipolar cycloaddition reaction, the interference between the relatively labile N-hydroxymethyl units and the reactants can be considered as negligible. The concept of our approach first describes a method to introduce pendant alkyne-moieties into the MF-resins materials, and subsequently the use of these sites for subsequent azide/alkyne-“click”-reactions. This publication will deal mostly with the analytical and chemical issues of this process, focusing on the analytics of the underlying multinuclear-triazine moieties by LC-ESI-TOF methods.



* Oligomeric structures were proved by HPLC-ESI-TOF-MS methods.

SCHEME 1 Preparation of melamine-formaldehyde condensates modified via "click"-chemistry using octylazide (**1**), benzylazide (**2**), thymineazide (**3**), and triacetondiamine (**4**).

EXPERIMENTAL

Materials and Measurements

All chemicals were purchased from Sigma Aldrich and used without further purification. All solvents, which were used for the workup procedures, were distilled prior to use. Tetrahydrofuran (THF) and toluene were pre-dried over potassium hydroxide for several days and freshly distilled from benzophenone-sodium under an argon atmosphere prior to use. Melamine, formaldehyde (45wt% aqueous solution), and *p*-toluenesulfonic acid were provided from Dynea Austria GmbH. n-Octyl azide (**1**), (azidomethyl) benzene (**2**), and 1-(6-azido-hexyl) thymine (**3**) were prepared as described in the Supporting Information. Benzyl bromide (HPLC-grade) and sodium hydroxide were purchased from Fluka. Thin layer chromatography was carried out on commercial Merck plates.

^{1}H- and ^{13}C-NMR spectra were performed on a Varian Gemini 2000 (200 and 400 MHz); FT-NMR spectrometer using MestRec-C (4.9.9.6) software for data interpretation. Chloroform ($CDCl_3$) and (DMSO-d_6) were used as solvents, and tetramethylsilane (TMS) as an internal standard.

IR-spectra were recorded on a Bruker Vertex 70 FTIR spectrometer equipped with a golden gate unit and a RT DLa TGS detector using OPUS (6.5) software for data evaluation.

HPLC-ESI-TOF measurements were performed on a *Elite LaChrom* HPLC by Hitachi VWR equipped with an autosampler, a quaternary gradient pump, a degasser, a diode array detector (UV-DAD) and a column oven with temperature control (temperature = 25 °C). The LC-separation was carried out on a Waters Nova Pak C18 column, 60 Å, 4 µm, dimension 3.9 mm × 150 mm, using 0.019 M ammonium acetate/H2O (solvent A) and 0.019 M ammonium acetate/methanol (Solvent B) as solvents. The flow rate was 0.4 mL/min with a linear gradient: 1/ 99 to 20/80 within 40 min, sample injection was 5 µL of 0.2 mg/mL. The output of the HPLC separation was directly introduced into the ESI source of the Bruker Daltonics micrOTOF time-of-flight LC-MS system. Spectra were recorded in the positive mode with an accelerating voltage of 4.5 kV, a transfer line with 190 °C and a scan range of 50–1000 m/z. The spectra were processed on Bruker Daltonics ESI compass 1.3 for micrOTOF (Data Analysis 4.0). Samples were prepared by dissolving 20 mg of the resin mixture in 10 mL 20/80; H_2O/methanol. Then 100 µL of this solution were diluted with 1 mL of 20/80; H_2O/methanol used for the LC-ESI-TOF measurements.

General Procedure of the Synthesis of the Organic Azides (1 - 4)

The general synthesis of the azides 1–3 is described in the Supporting Information.[19]

Synthesis of 4-Azido-N-(2,2,6,6-tetramethyl piperidin-4-yl)benzamide (4)

A solution of 4-amino-2,2,6,6-tetramethylpiperidine (103 mg, 0.659 mmol) in 20 mL of dry dioxane was added dropwise to a cooled solution (10 °C) of 4-azido-benzoyl chloride (120 mg, 0.661 mmol) in 10 mL dioxane and the temperature was kept below 15 °C. The reaction mixture was stirred for 16 h at room temperature. Subsequently,

the formed pale yellow precipitate was filtered off, thoroughly washed with dioxane and dried under reduced pressure. Yield: 183 mg (92%). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.44 (d, 1H; CO–NH), 7.88; 7.86; 7.17; 7.15 (AB, 4H, Ar) 4.28 (br s, 1H, N–H), 1.88, 1.85, (d, 2H, CH_2) 1.32, 1.27 (d, 2H, CH_2), 1.39 (d, 12H, CH_3), 1.57 (t, 1H, NH–CH2). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 165.2, 142.7, 131.3, 129.7, 119.3, 66.2, 56.2, 30.7, 25.19

Structural identification via LC-ESI-TOF

The fully alkyne substituted melamine ring can be represented by the formula M_1P_6 where M represents the melamine ring; $M_{1,2,3}$ correspond to the mono-, di- and trimeric melamine-species within the compound; F is the number of hydroxymethyl units (-CH_2OH) on the three primary amino groups, P is the number of methylene propargyl ether units (H-C≡C-CH_2-CH_2-), Me the number of methoxy units (-OCH_3), and E, E` represent the number of methylene (-CH_2-) and methyleneoxy units (-OCH_2-), respectively. Other compounds having multiple numbers of F and P or Me end-groups can be represented by the general formula $M_aF_bE_cE'_dP_eMe_f$. In addition, the modified precondensates via azide/alkyne "click"-reactions also contain dimeric and oligomeric species formed by subsequent condensation reactions. The "clicked"-structures have the general formula $M_aF_bE_cE'_dP_eMe_f$ -(TA_g, BA_h, OA_i, TD_j), where *TA* is 1-(6-(5-methyleneoxy-1*H*-1,2,3-triazol-1-yl)hexyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione, *BA* is 1-benzyl-5-methyleneoxy-1*H*-1,2,3-triazole, *OA* is 5-methyleneoxy-1-octyl-1*H*-1,2,3-triazole and TD is 4-(4-(methyleneoxy)-1*H*-1,2,3-triazol-1-yl)-*N*-(2,2,6,6-tetramethylpiperidin-4-yl)benzamide.

Functionalization of melamine-formaldehyde with a pendant alkyne-group

An aqueous solution of 44 % formaldehyde (3.7 g, 0.0555 mol) was heated to 44°C ± 1.0°C. After correction of the pH-value to slightly basic conditions (pH = 9.3±0.5) using a 14 wt% NaOH solution, melamine (2.5 g, 0.0198 mol) was added within 15 min under vigorous stirring (F/M ratio = 2.70). The temperature of the reaction mixture was raised to 70°C whereupon a clear solution was obtained. Subsequently, propargyl alcohol (4.84 g, 0.0863 mol = 86.3 mmol) was swiftly injected followed by the addition of methanol (13.8 g, 0.431 mol). The reaction temperature was lowered to 60°C, and the condensation reaction was initiated by adjusting the pH to slightly acidic conditions (pH = 5.3±0.5) using *p*-toluenesulfonic acid after 30 min. Furthermore, the mixture was cooled to room temperature and the resin was stabilized by adjusting the pH value to basic conditions (pH = 9.2±0.5). Methanol and/or unreacted propargyl alcohol was removed using a rotary evaporator through a two-step procedure; starting at 400 mbar at room temperature and finally at 50 mbar and 70°C.

Quantification of acetylene functionalized MF condensate

The qualitative and quantitative analysis of the acetylene functionalized MF condensates (**5**-**13**, Scheme 2) was achieved via analysis of the LC-traces and ESI-TOF-spectra. All spectra depicted the characteristic ion peaks derived from the investigated MF condensates similar to the LC-method described for the methoxylated MF resins, the ones with alkyne-groups and the others without those. After identification of the respective species inside the LC peaks by MS, the quotient of the area of former MF condensates and the area of all MF condensates corresponding to the mol% alkyne functionalized MF (mol% P) condensates were calculated as shown in eq. 1 (A_i represents the sum of the calculated absolute areas of all the structures bearing acetylene groups; A_t is the total area of the whole MS-trace).

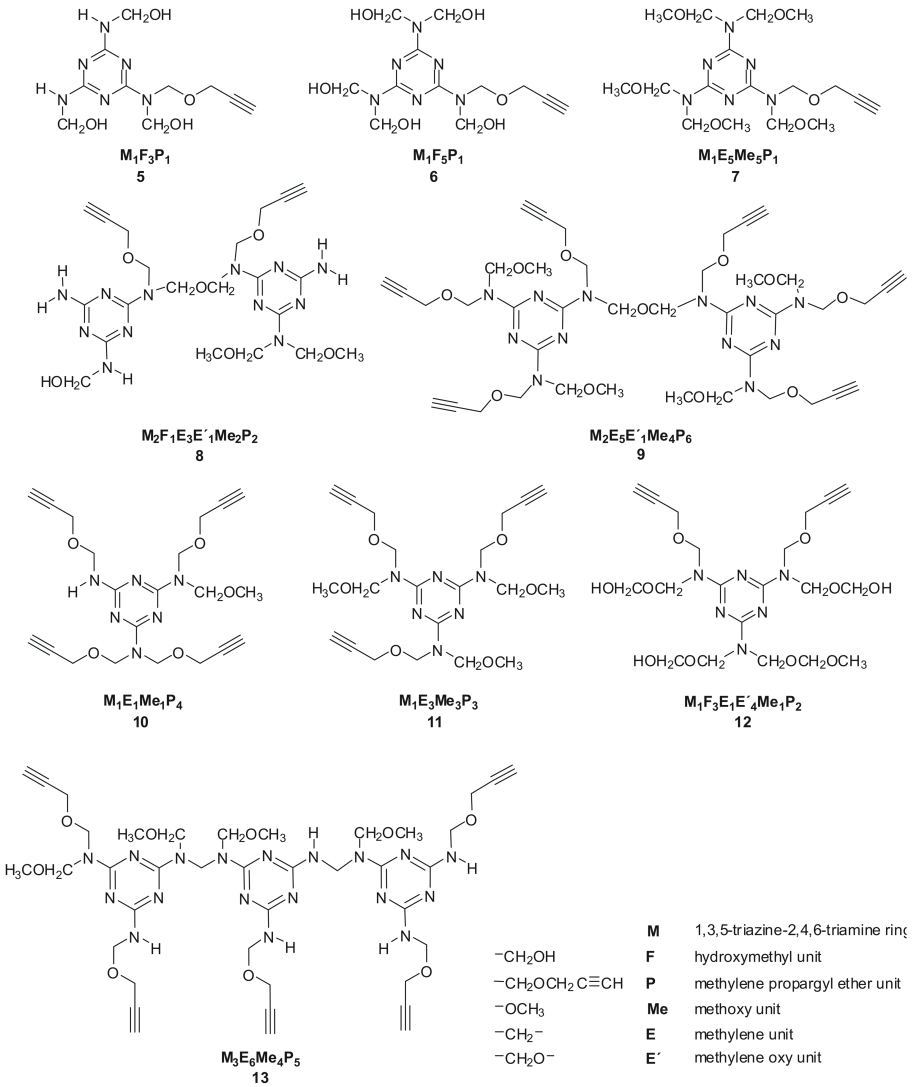
$$mol\%\,P = \frac{\sum A_i}{A_t} \times 100 \qquad (1)$$

The individual structures with acetylene functionality can be monitored via the ESI-TOF MS detector and the area of the new peaks appearing in the MS-trace is correlated to the sum of the total intensities of *m/z* species, which appear at the corresponding retention time. However, the area under these peaks shows not only the intensities of alkyne-modified species but also species without alkyne-species. Therefore, the quotient of the absolute area (A_i) of the modified species is calculated by dividing the sum of their intensities (I_n; multiplied unit of the number of the alkyne-groups (n_i) in the molecule) by the total sum of the intensities (I_t) and correlating it with the area under peak A*p* as illustrated in eq. 2.

$$A_i = \frac{\sum n_i I_n}{I_t} \times A_p \qquad (2)$$

Azide/alkyne "click"-reaction of melamine-formaldehyde condensate

The azide/alkyne-"click"-synthesis of the modified resins $M_aF_bE_cE'_dP_eMe_f$ -(TA_g, BA_h, OA_i and TD_j) was conducted under Cu(I)-mediated conditions according to the following procedure: the melamine-formaldehyde resin functionalized with terminal alkyne-groups (107 mg, ~3.9mol% of alkyne-moieties as proven by LC-ESI-TOF), DIPEA (0.3 ml, 1.722 mmol), the respective azides (i.e.: TD (4) = 107 mg, 0.355 mmol) and Cu(I)Br (10 mg, 0.071 mmol) were dissolved in deaerated methanol (9 ml) and stirred at 70°C for 12-24 h. These mass spectra clearly prove the formation of melamine-species, modified with one, two or more propargylic units, as visible by comparing the measured ESI-spectra with the corresponding simulated isotope-patterns of selected ions. A selection of chemically different melamine species resulting from this type of reaction is shown in Scheme 2. Thus the formation of mononuclear, as well as bi- and trinuclear species (i.e.: species with one, two and three melamine-units), bearing one- or several alkyne-moieties can be proven within the reaction mixture. For simplification purposes, an extended specific nomenclature is used to specify the individually present moieties on the respective triazine-moieties. Subsequently, the reaction mixture was filtered-off to remove Cu(I)-species, methanol was removed using a rotary evaporator by a two-step procedure starting at 400 mbar at room temperature and finally at 50 mbar and 70°C to furnish a viscous yellow-orange product. Traces of copper were then removed by re-dissolving the crude product in methanol and filtration of the resulting material over a short column of alumina.



SCHEME 2. Alkyne-modified MF resin compounds resulting from addition of 86.3 mmol of propargyl alcohol as identified characterized and quantified via HPLC- ESI-TOF.

RESULTS AND DISCUSSION

The basic synthetic methodology consists of (**a**) the reaction of melamine with formaldehyde together with the addition of propargylic alcohol and (**b**) the azide/alkyne-"click"-reaction furnishing the finally functionalized MF-resin with the azide 1–3 (see Scheme 1). As the co-condensation of melamine with formaldehyde yields a multitude of different species, a detailed individual analysis of both steps is required, thus enabling to specify the individually modified melamine species. To this latter purpose, the extensive use of LC-ESI-TOF methods is envisioned as a reasonable separation of the individual melamine-species can be achieved by LC-methods using mass spectrometry as identification to nail down their detailed chemical structure.[1-5]

Synthesis of MF-resins with pendant alkyne-moieties

The introduction of pendant alkyne-moieties into MF-resins was achieved by addition of propargylic alcohol to a slightly basic mixture of melamine and formaldehyde. Individual LC-traces of the finally obtained resin were monitored with increasing amount of the added propargylic alcohol. Figure 1 shows the LC-traces of MF-resins modified with increasing amounts of propargylic alcohol (0 to 86.3 mmol), in mixture with methanol. Clearly, significant changes of the LC-traces at elution times between 20-30 minutes are visible (marked in grey) allowing to assume that new species are formed in comparison to the native, unmodified resin material. In order to achieve a more detailed picture of the newly generated species, each peak was analyzed directly via ESI-TOF-spectrometry allowing to collect an individual mass spectrum of each species, together with the isotopic pattern of the respective molecule (see Figure 2).

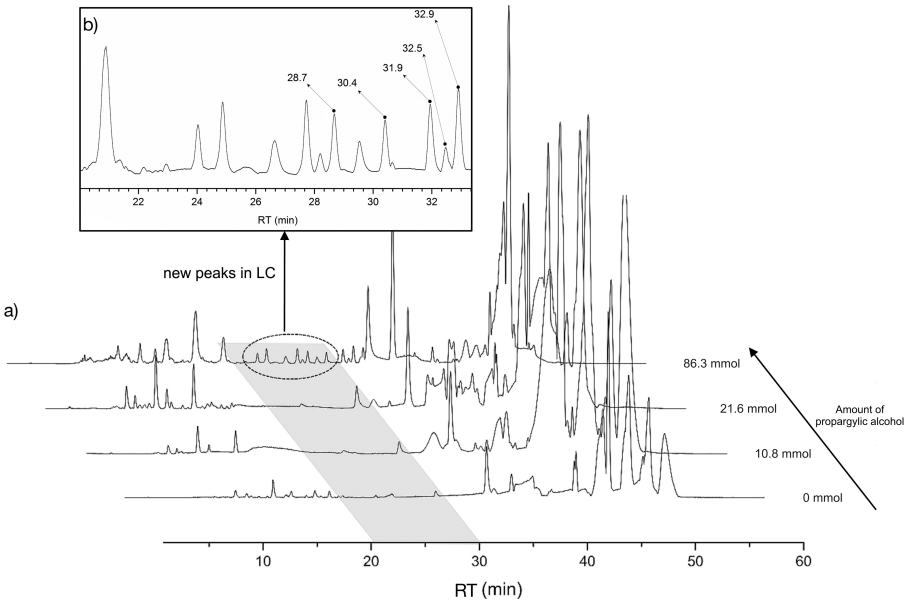


FIGURE 1. (a) LC-traces of amino- resins prepared with increasing amounts of propargylic alcohol during the condensation reaction with MF resin, and (b) expanded region of the newly produced peaks by addition of 86.3 mmol of propargylic alcohol.

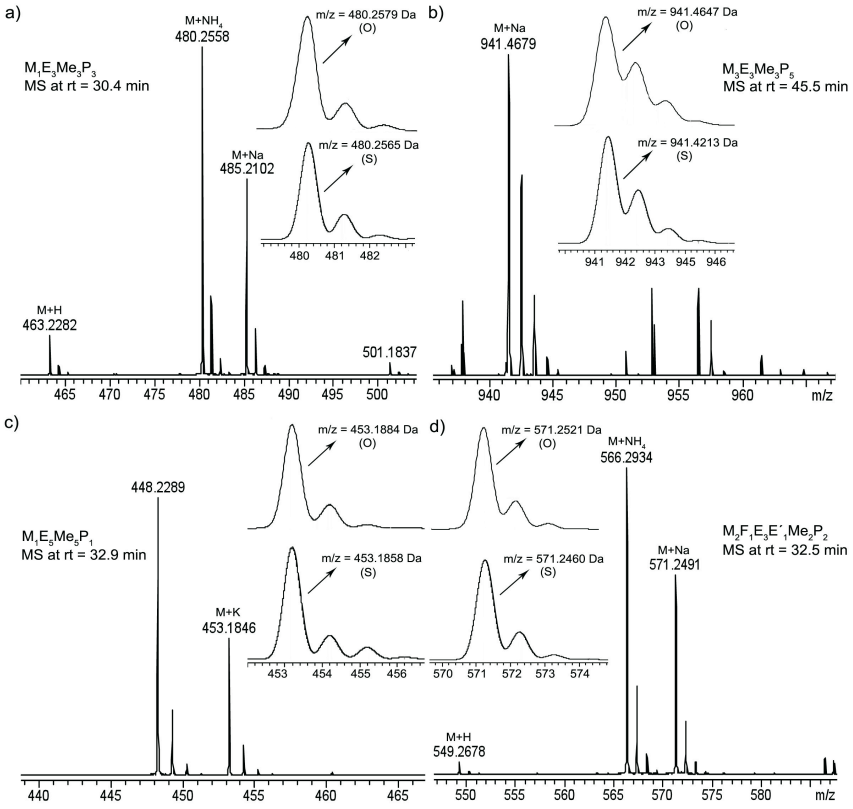


FIGURE 2. MS-spectra of the unimer ($M_1E_3Me_3P_3$), the dimer ($M_2F_1E_3E'_1Me_2P_2$) and the trimer ($M_3E_3Me_3P_5$) of the MF resin bearing alkyne-units. Main traces: measured and (inserts) simulated isotope-patterns using Bruker isotopic-pattern software.

Thus, the designations used in Scheme 2 are used throughout the paper, in order to achieve a species-specific picture of the chemical composition. The respective ions are collected in Table 1, with most of the ions desorbed as $[MH]^+$, M^+, $[MNH_4]^+$ or $[MNa]^+$-species. When comparing the measured with the simulated mass peaks, they show an excellent match of the individual species, with errors ranging from 2.6 to 138.1 ppm, indicating the true formation of the expected species. However, those with increased level of substitution bearing multiple alkyne-units have been observed at retention times rt= 30-37 min, e.g., $M_1E_1Me_1P_4$ (**10**), $M_1E_3Me_3P_3$ (**11**) and $M_1F_3EE'_4Me_1P_2$ (**12**). Compounds with two melamine rings ($M_2F_1E_3E'_1Me_2P_2$ and $M_2E_5E'_1Me_4P_5$) formed by ether bridging are detected at rt= 30-33 min.

TABLE 1. Molecular Masses Found in ESI-TOF Mass Spectra Received by Analysis of the Modified MF Resin with 86.3 mmol Propargylic Alcohol

Entry	Formula	Compounds[a]	HPLC rt[b] (min)	ESI-MS-TOF			Ionized species
				Observed *m/z* (Da)	Simulated *m/z* (Da)	Error (ppm)	
1	$C_{10}H_{16}N_6O_4$	M_1F_3P (5)	11.1	307.155	307.113	-138.1	$[MH]^+$
2	$C_{12}H_{20}N_6O_6$	M_1F_5P (6)	28.7	345.167	345.152	-43.7	$[MH]^+$
3	$C_{17}H_{30}N_6O_6$	$M_1E_5Me_5P_1$ (7)	32.9	453.185	453.186	2.6	$[MK]^+$
4	$C_{21}H_{32}N_{12}O_6$	$M_2F_1E_3E'_1Me_2P_2$ (8)	32.5	571.252	571.246	-10.7	$[MNa]^+$
5	$C_{40}H_{54}N_{12}O_{11}$	$M_2E_5E'_1Me_4P_6$ (9)	31.9	878.418	878.403	-17.5	$[M]^+$
6	$C_{21}H_{26}N_6O_5$	$M_1E_1Me_1P_4$ (10)	36.6	465.175	465.186	22.6	$[MNa]^+$
7	$C_7H_{14}N_6O_4$	$M_1E_3Me_3P_3$ (11)	30.4	480.258	480.257	-2.7	$[MNH_4]^+$
8	$C_{20}H_{32}N_{12}O_{10}$	$M_1E_1E'_4Me_1P_2$ (12)	33.8	534.252	534.270	34.1	$[MNH_4]^+$
9	$C_{39}H_{54}N_{18}O_4$	$M_3E_6Me_4P_5$ (13)	45.5	941.465	941.421	-46.1	$[MNa]^+$

[a]For structural details see Scheme. [b]rt: retention time in HPLC- chromatogram.

The compound $M_3E_6Me_4P_5$ (**13**) shows an example for a molecule with three melamine species with methylene bridging bearing many alkyne-units. It should be noted, however, that the substitution pattern of all compounds shown in Scheme 2 cannot be specified exactly, as the positions of all substituent on the melamine-species may vary in position on the amine-moieties of each melamine-species. Additionally another method to identify the formation of MF-compounds bearing alkyne functionality inverse-gated (IG) decoupled ^{13}C-NMR was used and compared with the pure, unmodified MF-resin (see Figure 3). Thus, the ^{13}C NMR spectra of the native MF-resins show distinct peaks at 72.2, 64.2 and 47-55 ppm corresponding to the NH**C**H$_2$OCH$_2$NH-, N(**C**H$_2$OCH$_3$)$_2$/NH(**C**H$_2$OCH$_3$), =N**C**H$_2$N=, =N**C**H$_2$NH-, -NH**C**H$_2$N= and -O**C**H$_3$ moieties, in accordance with literature.[2] After the addition of increasing amounts of the propargyl alcohol (A = 86.3 mmol; D = 107.8 mmol), the appearance of weak new resonances at 80.8, 76.3 and 70.15 ppm can be assigned to H-C≡**C**-CH$_2$-X, H-**C**≡C-CH$_2$-X and H-C≡C-**C**H$_2$-X, respectively, where X represents the linked triazine ring. Resonances at 84.01, 74.69 and 48.59 ppm are assigned to residual propargylic alcohol (H-C≡**C**-CH$_2$-OH, H-**C**≡C-CH$_2$-OH and H-C≡C-**C**H$_2$-OH).

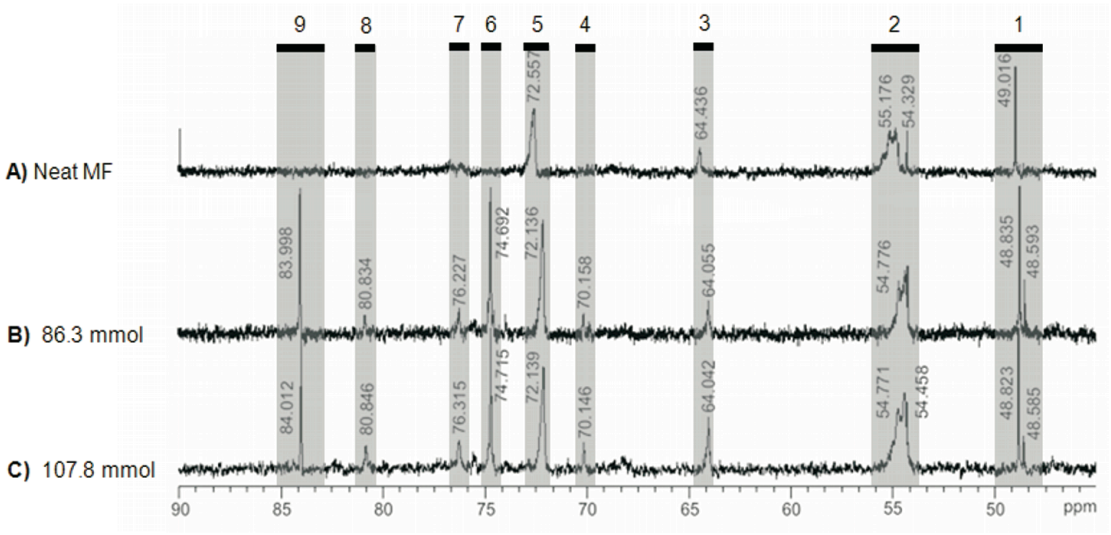


FIGURE 3. Expansion of inverse-gated decoupling of 13C-NMR spectra of the unmodified melamine formaldehyde resin (MF neat) and the amounts of propargyl alcohol added during the cocondensation process. (A = neat resin; B = 86.3 mmol; C = 4 107.8 mmol).

TABLE 2. 13C-NMR Integrals of MF-Resins Modified with Propargylic Alcohol (A 5 0 mmol, B 5 86.3 mmol, C 5 107.8 mmol), Measured with Inverse-Gated (IG) Decoupling 13C-NMR Method.

C-atom	Region	Chemical Shift (ppm)	Integration /^{13}C-NMR A	C	D
Ar-C	--	167.5	3.00	3.00	3.00
H-C≡C-CH_2-OH	8	84.0	--[a]	3.93	1.44
H-C≡C-CH_2-OH	5	74.7	--[a]	5.66	2.17
H-C≡C-CH_2-X	7	80.8	--[a]	0.30	0.36
H-C≡C-CH_2-X	6	76.3	--[a]	0.69	0.52
-NH(CH_2OCH_3)	--	72.1	4.65	5.60	3.28
-OCH_3	2	54.8	5.96	6.95	6.66
H-C≡C-CH_2-X	4	70.1	--[a]	0.24	0.32

[a]Signals were not observed in spectra.

At lower amounts of propargylic alcohol (10.8 mmol; 21.6 mmol) no distinct resonances for triazine-linked acetylenes could be detected, presumably by being below the detection limit of ^{13}C-NMR-spectroscopy for these species (detection limit ~ 5 %). In Table 2, the ratios of different carbons present per triazine in these resins were calculated, indicating the covalent attachment of the propargylic alcohol to the MF-resin in amounts of up to ~10 mol% with respect to the melamine-species. We feel (*vide infra*) that these values overestimate the amount of bound alkynes, as lower amounts (~ 3.9 mol%) are

TABLE 3. Quantification of Free Propargylic Alcohol Residues in the Purified MF Mixture, After the Cocondensation Process Using Inverse-Gated (IG) Decoupling 13C-NMR Spectroscopy.

Entry	Propargylic Alcohol (mmol)	^{13}C-NMR Quantification[a] Unreacted Propargylic Alcohol (mol%)	LC-ESI TOF MS Quantification Bound Alkyne Moieties (mol%)
1	86.3	3.5	3.9
2	43.1	--[b]	0.6

The incorporated alkyne-moieties were quantified via LC-ESI-TOF MS methods.[a]Determined via integration of signals observed in inverse-gated (IG) decoupling 13C-NMR spectra.[b]The chemical shifts of the signals that correspond to propargyl alcohol were not observed in the 13C-NMR spectra of the resins modified with 21.6–43.1 mmol of propargylic alcohol.

detected with the subsequent LC-ESI-TOF method. We, therefore, sought for another way to quantify the formed alkyne-species with increasing amount of added propargylic alcohol (see Table 3). Thus, the peak-areas of the respective peaks in the LC-trace specific for the alkyne-containing species were determined and divided by the overall-peak ratio of all LC-peaks. The presence of more than one alkyne-moiety in many of the formed molecules was taken into consideration. In this way, a rough quantification of the amount of alkyne-species in the respective resins could be obtained (see Table 3)

TABLE 4. Results and Reaction-Conditions of the Alkyne/Azide "Click"-Reaction Using Cu(I)Br and DIPEA as a Base in Methanol at 70 8C for 12–24 h.

Entry	Resin/Alkynes Units (mol%)[a]	Azide	Catalytic System CuBr/DIPEA[b] (mmol)	Time (h)	LC-ESI-TOF MS Triazole Species (mol%)[c]	LC-ESI-TOF MS Alkyne Residues (mol%)[d]
1	3.9	1	0.028/10.99	12	3.9	≤0.06[e]
2			0.014/10.99	12	3	≤0.06[e]
3			0.045	24	--[f]	≤0.06[e]
4			0.045/5.42	24	2.9	≤0.06[e]
5			0.021/10.99	24	3.2	≤0.06[e]
6			0.028[d]	24	--[f]	≤0.06[e]
7		2	0.085/7.74	24	2.7	≤0.06[e]
8			0.028/9.28	24	2.2	≤0.06[e]
9			0.045[d]	24	--[f]	≤0.06[e]
10		3	0.123/3.56	24	1.7	≤0.06[e]
11			0.075/2.32	24	--[f]	≤0.06[e]
12		4	0.071/2.32	24	--[g]	--[g]
13	0.60	1	0.021/8.51	24	≤0.06[e]	0.27
14		2	0.014/8.51	24	≤0.06[e]	0.15

DIPEA = N,N-diisopropylethylamine

[a] In mol % of alkyne-modified MF resins as determined via HPLC-ESI-TOF analysis.

[b] Cu(I)Br and DIPEA with respect to moles of alkyne-modified MF resins.

[c] Mol % of MF resin structures bearing triazol-moieties as determined via LC-ESI TOF.

[d] Pure Cu(I)Br used without DIPEA.

[e] Minimal detectable amount via this method.

[f] Triazole species were not observed in LC-ESI-TOF spectra.

[g] Quantification was not possible due to peak overlap in the MS-trace.

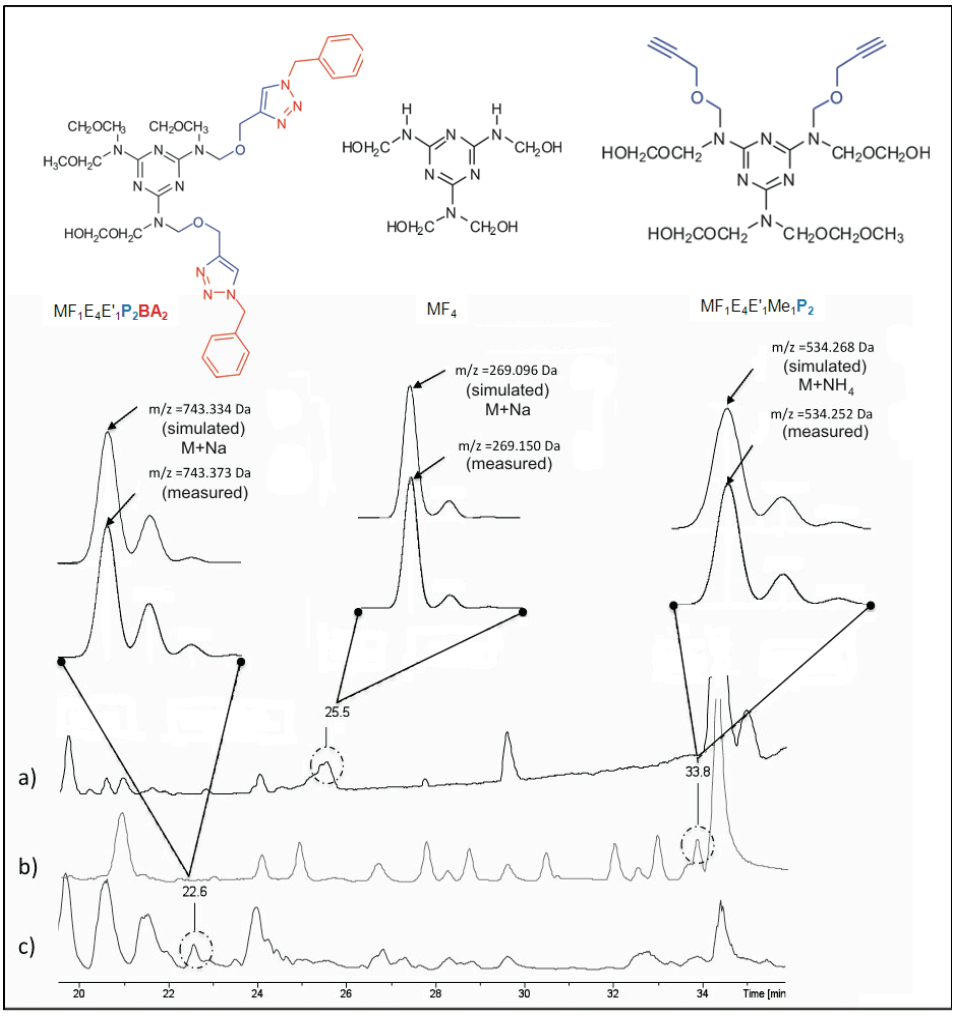


FIGURE 4. Molecular masses observed under LC-chromatograms of (a) neat MF resin, (b) alkyne-modified MF resin, and (c) the modification of propargylic MF with benzyl azide (BA, 2) via azide/alkyne"click"-reactions (Cu(I)Br/DIPEA; 0.028/9.28 for 24 h).

Thus, the addition of increasing amounts of propargylic alcohol (32.3, 43.1, 86.3 mmol) to the resin during condensation, lead to an increase of covalently-bound alkyne-species in amounts of 0.64 to 3.9mol%. With the lowest probed amount of propargylic alcohol (21.6 mmol) no alkyne-species were detected in the final melamine resin.

Azide/alkyne-"Click"-Reactions on MF-resins

Several alkyne-modified resins were then investigated in the azide/alkyne-"click"-reaction using the azides **1** – **4**. The azides shown contain either purely aliphatic or aromatic moieties (**1**, **2**) acting as model systems, whereas the functional azides (**3**) and (**4**) contain a hydrogen bonding moiety and a sterically hindered piperidine moiety. The latter in particular is an important functional moiety acting as photostabilisator, whose covalent binding to the amino resin is also of commercial interest. An overview on the used reaction conditions for the azide/alkyne-"click"-reactions is shown in Table 4. Thus, Cu(I)Br (~ 0.045 mmol with respect to the alkyne-moieties) was used in all reactions. Reactions were conducted with (see entries 1, 2, 4, 5, 7, 8, 10-14) and without (entries 3, 6, 9) the addition of diisopropylethylamine (DIPEA), detecting no click-reaction without the addition of DIPEA no "click"-reaction (see entries 3, 6, 9). Starting from a resin with ~ 3.9 mol% of alkyne moieties (Table 4, entries 1 - 12) the azides **1** and **2** yielded good results with respect to the formation of the desired triazole-species. Using the azide (**3**) (see entry 10) the formation of ~1.7 mol% of the "clicked"-species were detected, however, using a relatively large amount of Cu(I)-species. The azide (**4**) did not yield quantifiable amounts of the respective triazole-linked melamine-species. Starting from a resin with ~ 0.6 mol% of alkyne moieties (Table 4, entries 13, 14), the azides **1** and **2** did not yield detectable amounts of the respective covalently bound triazole/melamine species, presumably due to the detection limit of the LC-ESI-TOF method. Again, the qualitative analysis of the individually formed species was achieved by LC-ESI-TOF methods (see Figures 4). As an example, the newly formed species $MF_5Me_3BA_2$ can be detected at a retention time of 22.6 minutes (see Figure 4) upon comparison of the LC traces of the alkyne-modified MF-resin and the MF-resin after conducting the azide/alkyne-"click"-reaction with benzyl azide (**2**).

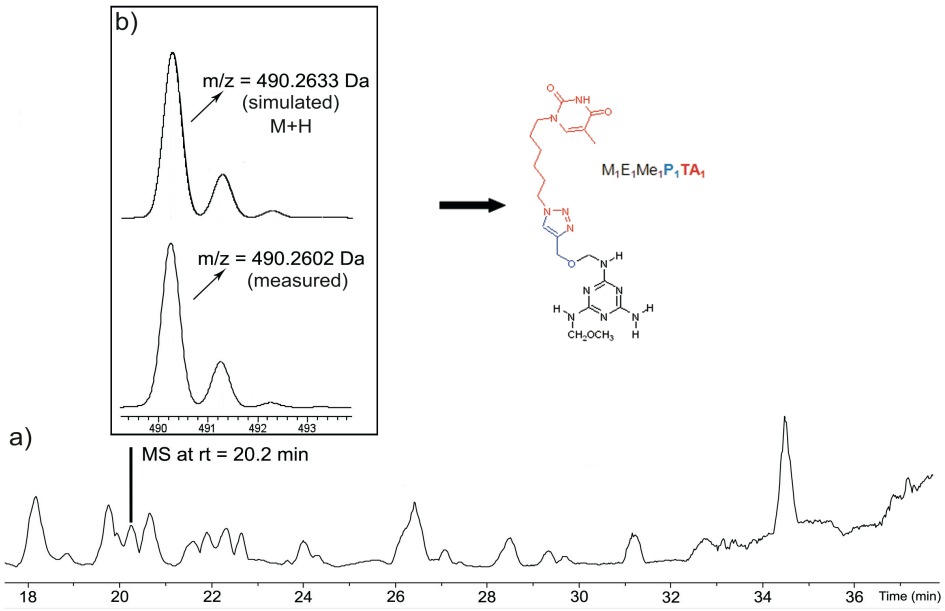


FIGURE 5. LC-chromatogram of modification of propargylic MF with 1-(6-azidohexyl) thymine (**3**) via azide/alkyne "click"-reaction CuBr/DIPEA; 0.028/10.99 for 12 h. Measured species were simulated using Bruker isotopic-pattern software.

A similar picture is seen in Figure 5, 6 and 7, showing the LC-traces and selected peaks of some selected species formed in the "click"-reactions. As an example Figure 5 indicates the formation of $M_1E_1Me_1P_1TA_1$-species in the LC-trace at 20.2 minutes as proven by comparing the detected with its simulated isotopic pattern. A full account of all species formed after the "click"-reaction of the alkyne-modified MF-resin (3.9 mol%) with the azide (**3**) is shown in Scheme 3 and Table 5. This Table shows all detected species containing the linked triazole with the pendant thymine-moiety (*TA*) linked to melamine-rings (M) within the MF-resin. The individual ions of the detected species are mostly desorbed as $[MH]^+$, M^+, $[MLi]^+$, $[MNH_4]^+$ or $[MNa]^+$-species, proving the formation of the expected triazole-substituted species. The formation of Li+- or K+-ions is not uncommon in MALDI- and ESI-TOF due to the high natural abundance due to the high natural abundance of these ions in the environment.

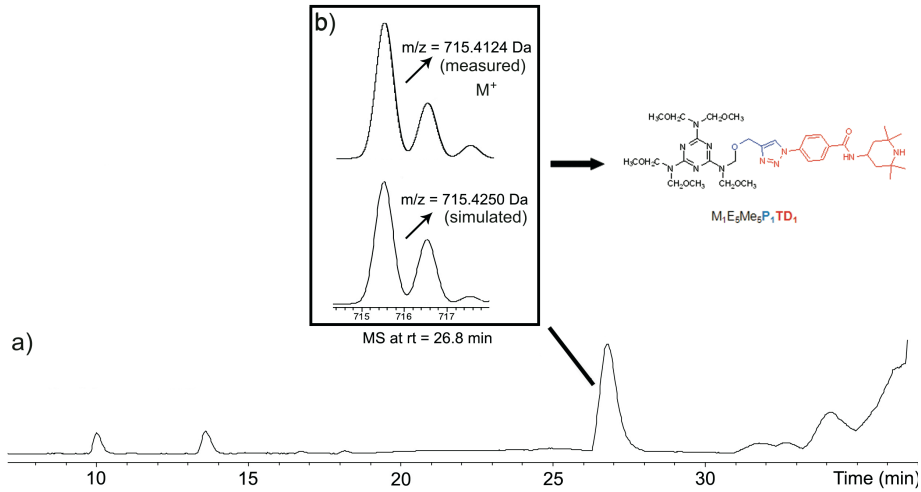


FIGURE 6. LC-chromatogram of post modification of propargylic MF with 4-azido-N-(2,2,6,6-tetramethylpiperidin-4-yl)benzamide (TD, **4**) via azide/alkyne-"click"-reaction (Cu(I)Br/DIPEA; 0.071/2.32 for 24 h). Measured species were simulated using Bruker isotopic- pattern software.

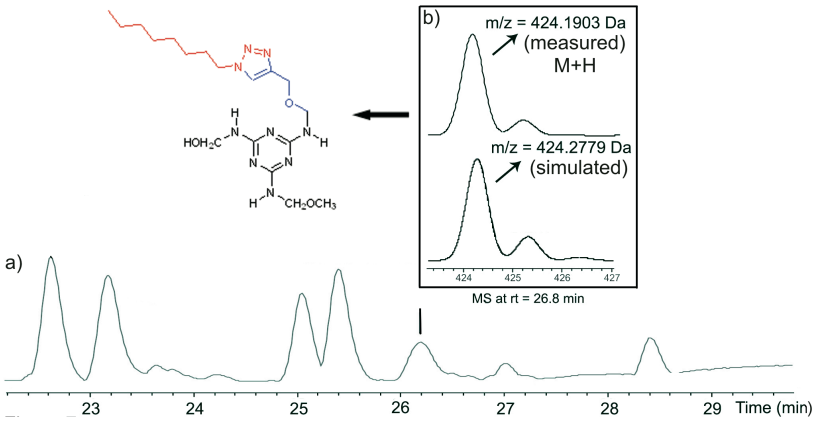
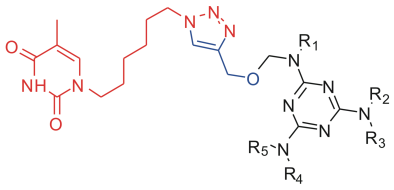


FIGURE 7. LC-chromatogram of post modification of propargylic MF with octyl azide (OA, **1**) via azide/alkyne"click"-reaction (CuBr/DIPEA; 0.028/10.99 for 12 h). Measured species were simulated using Bruker isotopic-pattern software.

Species containing one melamine ring and one thymine-moiety ($M_1P_1TA_1$, $M_1E_1Me_1P_1TA_1$, $M_1F_2P_1TA_1$, $M_1E_2Me_2P_1TA_1$, $M_1E_5Me_5P_1TA_1$, $M_1E_4Me_4P_1TA_1$, $M_1F_2E_2E'_2Me_2P_1TA_1$), two melamine (M) rings with one TA-moiety ($M_2E_3Me_2P_1TA_1$); one melamine ring with two thymine moieties (TA) ($M_2E_3Me_2P_1TA_1$) and species containing two linked thymine moieties ($M_1F_1E_1Me_1P_2TA_2$, $M_1F_3P_2TA_2$, $M_1E_3E'_1Me_3P_2TA_2$, $M_1E_4Me_4P_2TA_2$, $M_2E_1P_2TA_2$) could thus be detected unambiguously via ESI-TOF analysis by comparison of the detected with the simulated isotopic patterns. A special case concerned the analysis of the "click"-reaction of the alkyne-modified MF-resin (3.9 mol%) with the azide 4 (TD), where detection of linked species proved difficult. However (see Figure 6 and Table 6) a large variety of triazole-linked MF-species were found. Thus, the formation of the species $M_1E_5Me_5P_1TD_1$ (see Figure 6) at an elution time of 26.8 minutes was proven by comparing the detected with the simulated isotopic pattern. Again (see Table 6) the formation of species containing one melamine-ring with one triazole-linked triacetonediamine (TD)-species ($M_1E_2Me_2P_1TD_1$, $M_1P_1TD_1$, $M_1E_2E'_2Me_2P_1TD_1$, $M_1E_3E'_3Me_3P_1TD_1$, $M_1E_5Me_5P_1TD_1$, $M_1E_1E'_1Me_1P_1TD_1$, $M_1F_4P_1TD_1$, $M_1F_5P_1TD_1$) were found and their structures proven by comparison of the isotopic patterns. Similar analyses of isotopic patterns and ions were done for the azides 1 and 2 as demonstrated in the supplementary (tables S1 and S2).



$M_1P_1TA_1$	R^1- R^5 = H	(13)	$M_2E_3Me_2P_1TA_1$	R^1 - R^3 = H R^4 = $-CH_2OCH_3$ R^5 = $M_1E_2Me_1$	(20)
$M_1E_1Me_1P_1TA_1$	R^1 = $-CH_2OCH_3$ R^2- R^5 = H	(14)	$M_1F_1E_1Me_1P_2TA_2$	R^1-R^2 = -H R^3 = $-CH_2OH$ R^4 = $-CH_2OCH_3$ R^5 = $-P_1TA_1$	(21)
$M_1F_2P_1TA_1$	R^1- R^2 = $-CH_2OH$ R^3- R^5 = H	(15)	$M_1F_3P_2TA_2$	R^1= -H R^2-R^4 = $-CH_2OH$ R^5 = P_1TA_1	(22)
$M_1E_2Me_2P_1TA_1$	R^1- R^2 = $-CH_2OCH_3$ R^3- R^5 = H	(16)	$M_1E_3E'_1Me_3P_2TA_2$	R^1 = -H R^2-R^3 = $-CH_2OCH_3$ R^4 = $-CH_2OCH_2OCH_3$ R^5 = $-P_1TA_1$	(23)
$M_1E_5Me_5P_1TA_1$	R^1- R^2 = -H R^3- R^4 = $-CH_2OCH_2OCH_3$ R^5 = $-CH_2OCH_3$	(17)	$M_1E_4Me_4P_2TA_2$	R^1 - R^4= $-CH_2OCH_3$ R^5 = $-P_1TA_1$	(24)
$M_1E_4Me_4P_1TA_1$	R^1 = H R^2- R^5 = $-CH_2OCH_3$	(18)	$M_2E_1P_2TA_2$	R^1= $-M_1P_1TA_1$ R^2-R^5= -H	(25)
$M_1F_2E_2E'_2Me_2P_1TA_1$	R^1 = H R^2- R^3= $-CH_2OH$ R^4- R^5 = $-CH_2OCH_2OCH_3$	(19)			

SCHEME 3. Observed chemical structures of the alkyne-substituted melamine-species of mono-, di-substituted triazine rings after the "click"-reaction with 1-(6-azidohexyl) thymine (**3**).

TABLE 5. Alkyne/Azide "Click"-Reaction Using 0.123 mmol of Cu(I)Br and 3.56 mmol of DIPEA as Base in Methanol at 70 °C for 12 h, the MF Bearing Thymine Moieties were Fully Characterized Using LC/ESI-TOF.

				ESI-MS-TOF			
Entry	Formula	Compounds	HPLC Rt[a] (min)	Observed *m/z* (Da)	Simulated *m/z* (Da)	Error (ppm)	Ionized Species
1	$C_{18}H_{27}N_{11}O_3$	$M_1P_1TA_1$	18.2	446.234	446.237	7.1	$[MH]^+$
2	$C_{20}H_{31}N_{11}O_4$	$M_1E_1Me_1P_1TA_1$	20.2	490.264	490.263	-0.5	$[MH]^+$
3	$C_{20}H_{31}N_{11}O_5$	$M_1F_2P_1TA_1$	45.2	528.335	528.241	-178.9	$[MNa]^+$
4	$C_{21}H_{33}N_{11}O_6$	$M_1E_2Me_2P_1TA_1$	21.9	534.292	534.289	-5.1	$[MH]^+$
5	$C_{22}H_{35}N_{11}O_7$	$M_1E_5Me_5P_1TA_1$	15.8	637.330	637.330	-0.3	$[M]^+$
6	$C_{24}H_{39}N_{11}O_7$	$M_1E_4Me_4P_1TA_1$	40.5	621.340	621.335	-7.5	$[M]^+$
7	$C_{24}H_{39}N_{11}O_8$	$M_1F_2E_2E'_2Me_2P_1TA_1$	43.9	654.335	654.332	-4.6	$[MH]^+$
8	$C_{26}H_{41}N_{17}O_5$	$M_2E_3Me_2P_1TA_1$	46.5	672.413	672.354	-86.5	$[MH]^+$
9	$C_{26}H_{43}N_{11}O_9$	$M_1F_1E_1Me_1P_2TA_2$	48.3	839.401	839.438	45.0	$[MH]^+$
10	$C_{27}H_{45}N_{11}O_8$	$M_1F_3P_2TA_2$	48.8	855.372	855.433	72.0	$[MH]^+$
11	$C_{40}H_{62}N_{16}O_{10}$	$M_1E_3E'_1Me_3P_2TA_2$	15.8	944.509	944.517	9.1	$[MNH_4]^+$
12	$C_{28}H_{47}N_{11}O_9$	$M_1E_4Me_4P_2TA_2$	43.9	979.494	979.463	-32.4	$[MNH_4]^+$
13	$C_{37}H_{54}N_{22}O_6$	$M_2E_1P_2TA_2$	48.8	925.474	925.450	-26.6	$[MNa]^+$

[a] *rt: retention time in HPLC-chromatogram.*

The analysis of the individually newly formed triazole-species was accomplished in a similar way as described previously by integration of the LC-traces after identifying the specific triazole-species. A compilation of these quantitative results are shown in Table 4, indicating that a nearly quantitative reaction of the terminal alkynes to the respective triazoles can be achieved in the case of the azides **1**, **2** and **3**. Despite the fact that azide **4** shows a large amount of different covalently melamine-linked species, a significant amount could not be quantified via the respective LC-traces. Summed up, the presented method demonstrates the formation of covalently linked melamine-species, which for the first time allows the attachment of functional-moieties directly onto melamine-resins in a simple and quantitative manner.

TABLE 6 Alkyne/Azide "Click"-Reaction Using 0.071 mmol of Cu(I)Br and 2.32 of DIPEA as Base in Methanol at 70 °C for 24 h, the MF Bearing Triacetonediamine Benzamide Moieties (TD, 4) were Fully Characterized Using LC/ESI-TOF.

				ESI-MS-TOF			
Entry	Formula	Compounds	HPLC rt[a] (min)	Observed *m/z* (Da)	Simulated *m/z* (Da)	Error (ppm)	Ionized Species
1	$C_{27}H_{41}N_{11}O_4$	$M_1E_2Me_2P_1TD_1$	34.0	584.329	584.342	22.2	$[MH]^+$
2	$C_{23}H_{33}N_{11}O_2$	$M_1P_1TD_1$	16.7	502.326	502.298	-55.7	$[MLi]^+$
3	$C_{29}H_{45}N_{11}O_6$	$M_1E_2E'_2Me_2P_1TD_1$	24.8	643.401	643.355	-71.5	$[M]^+$
4	$C_{32}H_{51}N_{11}O_8$	$M_1E_3E'_3Me_3P_1TD_1$	45.8	756.486	756.356	-171.9	$[MNa]^+$
5	$C_{33}H_{53}N_{11}O_7$	$M_1E_5Me_5P_1TD_1$	26.8	715.424	715.413	-15.4	$[M]^+$
6	$C_{26}H_{39}N_{11}O_4$	$M_1E_1E'_1Me_1P_1TD_1$	42.8	569.368	569.319	-86.1	$[MNa]^+$
7	$C_{27}H_{41}N_{11}O_6$	$M_1F_4P_1TD_1$	16.6	615.332	615.324	-13.0	$[M]^+$
8	$C_{28}H_{43}N_{11}O_7$	$M_1F_5P_1TD_1$	19.9	663.341	663.369	42.2	$[MNH_4]^+$

[a] rt: retention time in HPLC-chromatogram.

CONCLUSION

The formation of alkyne-modified amino-resins made from melamine/formaldehyde resins and the subsequent azide/alkyne-"click"-reaction was probed leading to an efficient chemical modification of amino-resins. Alkyne-modified melamine-formaldehyde resins were prepared via a direct co-condensation approach using propargylic alcohol (21.6-86.3 mmol) as an additive. The LC-ESI-TOF method was used to analyze the individually formed chemical compounds in the resin up to molecular weights of ~1000 Dalton. Several alkyne-modified mono-, bi- and trinuclear melamine-species were identified, proving the covalent incorporation of alkyne-moieties in amounts of up to 3.9 mol%. At amounts below 21.6 mmol of propargylic alcohol, no incorporation of alkyne-moieties could be proven by LC-ESI-TOF, presumably due to the detection limit of the LC. Subsequent modification of the alkyne-modified resins was accomplished by reaction of functional azides (octyl azide (1), benzyl azide (2), 1-(6-azidohexyl) thymine (3) and 4-azido-*N*-(2,2,6,6-tetramethylpiperidin-4-yl)benzamide (4)) in the presence

of Cu(I)Br and DIPEA as a base. The formation of the respective triazolyl-modified MF-resins is proven by LC-ESI-TOF methods, indicating the successful covalent modification of the amino-resin with the azides 1–4. The method opens a simple chemical modification of amino-resins under aqueous conditions.

ACKNOWLEDGEMENT

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SUPPORTING INFORMATION

SYNTHESIS

1. Synthesis of (azidomethyl) benzene (**2**)

The general synthesis is described in literature.[6]

The azide derivative was synthesized by adding benzylbromide onto a 0.5 M solution of NaN_3 in DMSO. The reaction mixture was stirred at 40 °C for 48 h. The reaction was controlled by TLC for completion, and subsequently water was added to the mixture. The latter was extracted with ether three times, and the ether phase was washed with water and brine. The product was dried over Na2SO4. Yield: 2.52 g (92.2 %). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.42 -7.33 (m, 5H, Ar-H), 4.35 (s, 2H, Ar-CH2-N_3).

2. Synthesis of 1-(6-azidohexyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**3**)

The general synthesis is described in literature.[7]

1-(6-bromohexyl)-5-methylpyrimidine-2,4(1H,3H)-dione (1.5 g, 5.2 mmol) and sodium azide (0.41g, 6.2 mmol) were refluxed in acetonitrile for 18 h. Consequently, the solid was filtered, and the solution was concentrated to a gum, which slowly crystallized. The material was recrystallized from water. Yield 1.1 g (83 %). ^{1}H-NMR provided in Figure S5.

3. Synthesis of 4-azidobenzoic acid (**4a**)

The general synthesis is described in literature.[8]

To a suspension of 4-aminobenzoic acid (4.5 g, 33 mmol) in water (25 mL) in a 2 L-round bottom flask was added concentrated HCl (5.6 mL) dropwise while the mixture was vigorously stirred. After the addition has been finished, the reaction mixture was cooled down to 0 °C with an ice-salt-mixture. A solution of $NaNO_2$ (2.3 g, 33 mmol) in water (10 mL) was added slowly (about 30 min) via dropping funnel. The color of the mixture changes to yellow-orange during the addition. Subsequently, a solution of NaN_3 (2.14 g, 33 mmol) in water (25 mL) was added slowly whilst the mixture was vigorously stirred, whereby an enormous foam formation could be observed. The cooling bath was removed and stirring was continued for 90 min and afterwards a 100 mL water and 125 mL ethylacetate were added. The phases were separated via a separating funnel, and the water phase was extracted with 50 mL ethylacetate twice. The organic phase was washed with 1 N NaOH (40 mL). The water phase was acidified with 1 N HCl (80 mL), whereby a yellow solid precipitates. During the acidification ethylacetate (150 mL) was added in portions, which solves the yellow solid.

The organic phase was separated, and the unified organic phases were then dried over $Na2SO_4$, filtered and concentrated via vacuum distillation at room temperature to furnish a yellow solid. Yield: 4.98 g (92 %). ^{1}H-NMR (400 MHz, CDCl3, δ, ppm): 8.11, (d, 2H, 3J = 8.62 Hz), 7.11 (d, 2H, 3J = 8.63 Hz) 13C-NMR (100 MHz, CDCl3, δ, ppm): 145.8, 132.2, 125.7, 119.1. IR: 2813, 2541, 2101, 1672, 1600, 1577, 1507, 1424, 1316, 1282, 1177, 1138, 1122, 934, 856, 765.

4. Synthesis of 4-azidobezoylchloride (**4b**)

The synthesis was accomplished according to literature with minor changes.[9]

4-Azidobenzoic acid (1 g, 6.13 mmol) was added to fresh distilled thionylchloride (22.2 mL, 306 mmol) and refluxed for 4 h at 75 °C. When the reaction has, finished the excess of thionylchloride was removed under vacuum. The achieved brown solid was dried using high vacuum. Yield: 1.1 g (99 %). IR: 3098, 2401, 2252, 2119, 1737, 1594, 1498, 1417, 1306, 1282, 1208, 1171, 1121, 876, 837, 815, 804, 726, 696, 642.

FIGURES

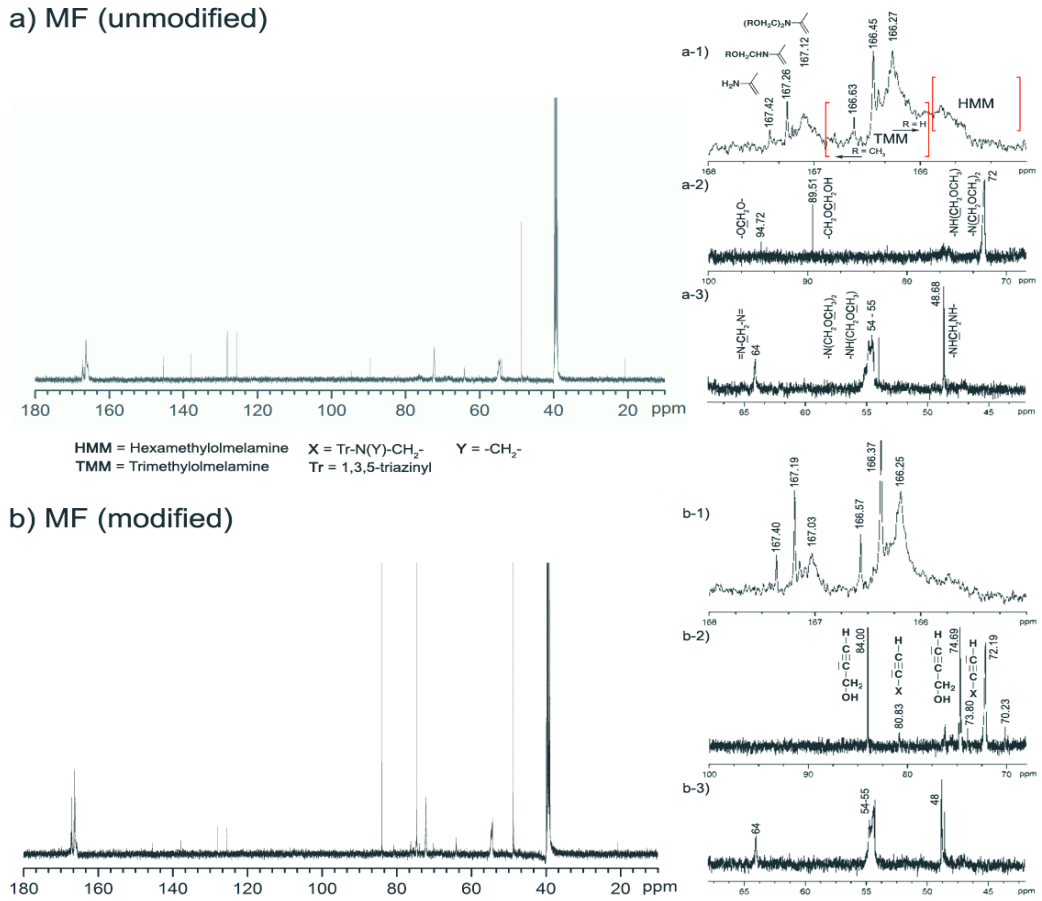

FIGURE S1. Inversed gate decoupling (^{13}C-NMR) spectra of; (a) the unmodified melamine formaldehyde resin (MF neat) and; (b) the alkyne-terminated melamine formaldehyde resin.

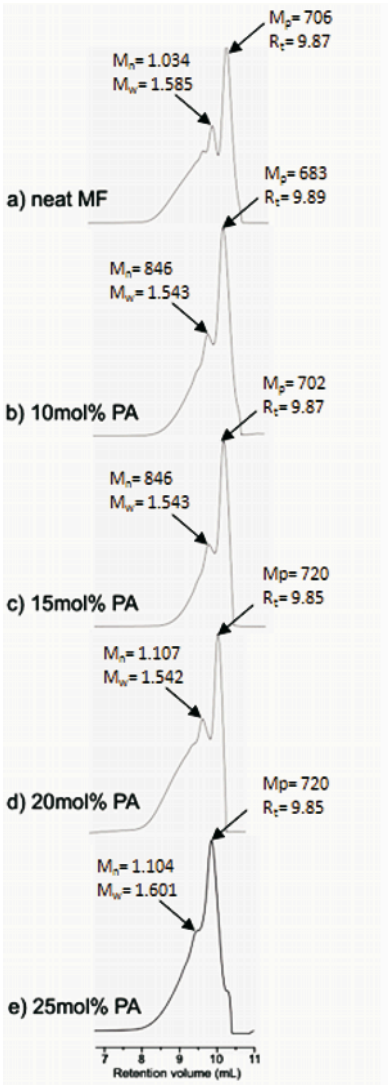

FIGURE S2. GPC-traces of neat MF resin and those modified ones with different amounts of propargyl alcohol (0-86.3 mmol).

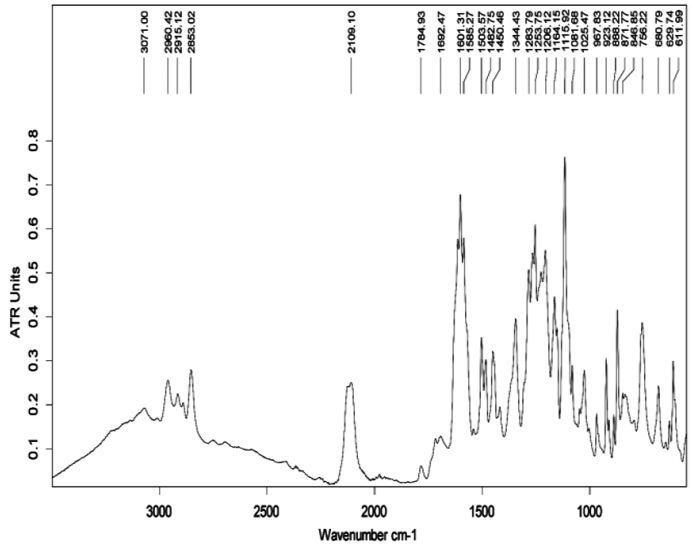

FIGURE S3. IR spectrum of monomer TD (**4**), showing the presence of the azide band at 2109 cm^{-1}.

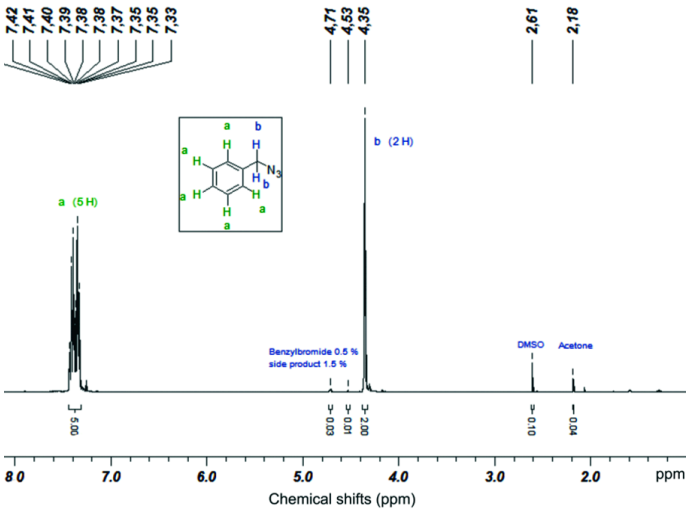

FIGURE S4. ^{1}H NMR spectrum of (azidomethyl) benzene (**2**).

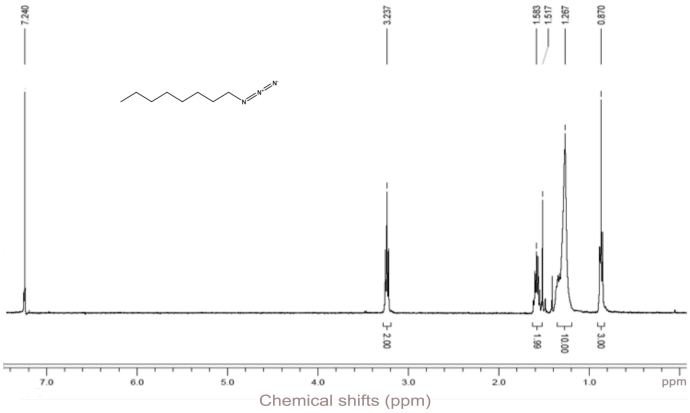

FIGURE S5. ^{1}H NMR spectrum of n-octyl azide (**1**).

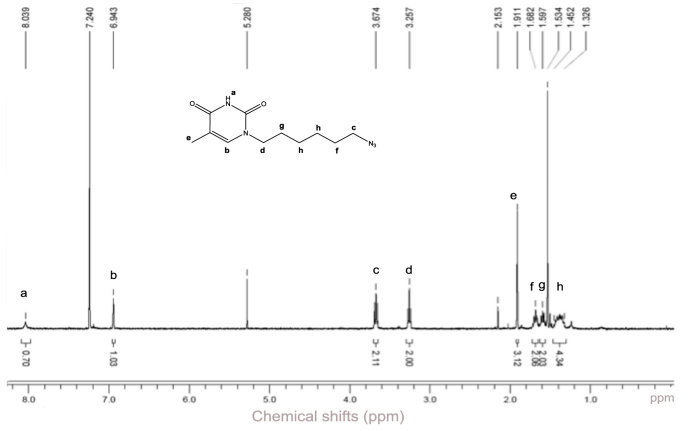

FIGURE S6. ^{1}H NMR spectrum of 1-(6-azidohexyl)thymine (**3**).

Table S1. Alkyne/azide "click"-reaction using 0.085 mmol of Cu(I)Br and 7.74 of DIPEA as base in methanol at 70 °C for 24 h, the MF bearing benzyl moieties (BA, 2) were fully characterized.

				ESI-MS-TOF			
Structure	Formula	Compounds	HPLC rt (min)	Observed m/z (Da)	Simulated m/z (Da)	Error (ppm)	Ionized Species
1	$C_{14}H_{17}N_9O$	MBA	19.2	328.161	328.163	5.790	M+H
2	$C_{16}H_{21}N_9O_2$	MFMeBA	21.7	372.186	372.189	8.598	M+H
3	$C_{18}H_{25}N_9O_3$	MF_2Me_2BA	24.1	416.210	416.215	12.734	M+H
4	$C_{20}H_{29}N_9O_4$	MF_3Me_3BA	27.2	460.239	460.242	5.866	M+H
5	$C_{25}H_{28}N_{12}O_2$	MBA_2	28	529.250	529.253	5.479	M+H
6	$C_{26}H_{30}N_{12}O_3$	$MFBA_2$	27.7	559.262	559.264	3.934	M+H
8	$C_{28}H_{34}N_{12}O_4$	MF_2MeBA_2	26.7	620.296	620.316	33.692	M+H
9	$C_{27}H_{32}N_{12}O_3$	MF_2MeBA_3	29.6	573.279	573.282	5.407	M+H
10	$C_{33}H_{44}N_{12}O_7$	$MF_5Me_3BA_2$	21.5	743.374	743.335	-52.601	M+Na
11	$C_{31}H_{38}N_{18}O_4$	MF_2MeBA_5	21.5	756.364	756.296	-89.912	M+K

Table S2. Alkyne/azide "click"-reaction using 0.028 mmol of Cu(I)Br and 10.99 mmol of DIPEA as base in methanol at 70 °C for 12 h, the MF bearing octyl moieties (OA, 1) were fully characterized.

				ESI-MS-TOF			
Entry	Formula	Compounds	HPLC rt (min)	Observed m/z (Da)	Simulated m/z (Da)	Error (ppm)	Ionized Species
1	$C_{15}H_{27}N_9O$	MOA_1	15.2	388.197	388.263	170.5	M+H
2	$C_{16}H_{29}N_9O_2$	$MFMe_2OA_1$	20.2	402.234	402.254	51.7	M+Na
3	$C_{17}H_{31}N_9O_3$	$MF_1Me_2OA_1$	23.4	432.244	432.293	113.1	M+Na
4	$C_{20}H_{37}N_9O_6$	$MF_2Me_2OA_1$	24.5	416.262	416.249	-29.8	M+Na
5	$C_{19}H_{35}N_9O_5$	MOA_1	45.6	460.289	460.276	-28.2	M+K
6	$C_{25}H_{47}N_9O_7$	MF_4OA_1	25.1	608.349	608.400	83.8	M+Na
8	$C_{21}H_{39}N_9O_5$	$MF_3Me_3OA_2$	30.1	515.341	515.290	-99.9	$M+NH_4$
9	$C_{18}H_{33}N_9O_3$	$MF_2Me_2OA_3$	26.8	424.278	424.190	-206.5	M+H

3.2 Multidimensional Characterization of α,ω-Telechelic Poly(ε-Caprolactone)s via Online Coupling of 2D Chromatographic Methods (LC/SEC) and ESI-TOF/MALDI-TOF-MS

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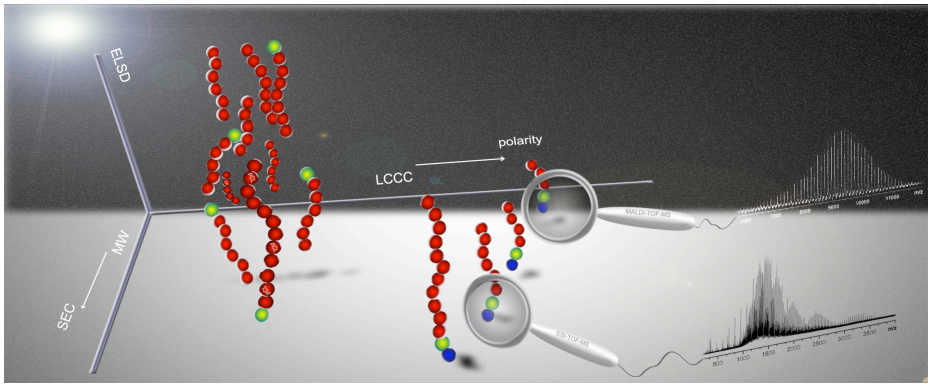

ABSTRACT: The focus of the current study is the development of chromatographic methods to understand the end-group modification of poly(ε-caprolactones) (pCLs) prepared via azide/alkyne "click" chemistry. In order to prove the efficiency of the "click" reaction with increasing molar mass of the pCLs, a detailed characterization method via several multidimensional chromatographic techniques (2D LC/SEC, coupled to mass spectrometry) was developed. In particular, we have investigated the separation of low molar mass pCLs ($M_n \leq 35\,000$ g·mol^{-1}) using a gradient elution chromatographic method (eluent: ACN/THF) monitoring the end-group functionalization of the separated polymers online via ESI-TOF and/or MALDI-TOF mass spectrometry, the latter coupled via a transfer module sprayer (TM sprayer). Because of the poor solubility of high molar mass pCLs (M_n above 35 000 g·mol^{-1}), a second chromatographic method was employed, enabling the separation of end-group-functionalized pCLs up to a molar mass of 115 400 g·mol^{-1}. Based on the critical solution conditions of the polymers, different solvent compositions and chromatographic columns were investigated in an isocratic mode to determine the critical conditions of α,ω-dihydroxy-telechelic pCLs. Finally, the critical conditions were optimized on a silica gel modified aminopropyl column (NUC-NH_2) using a solvent mixture ratio of DCM/THF of 92/8 at 35 °C. A combination of the developed LCCC methods with SEC revealed the chemical composition as well as the molar mass of all of the modified pCLs, from the low molar mass polymers up to M_n = 115 400 g·mol^{-1}.

Introduction

Telechelic poly(ε-caprolactone)s (pCLs) which are prepared via ring-opening polymerization (ROP) of lactones1 constitute versatile polymers used widely in pharmaceutical and medical sciences due to their excellent mechanical properties, biodegradability, and biocompatibility.[1−3] These attributes make them important materials in biopharmaceutical research that use nanocarriers[4,5] or microcapsules[6,7] based on self- assembled pCL-block copolymers (BCPs) showing a stimuli-response release of their encapsulated contents.[8,9] Based on ROP-based strategies, a large variety of dendritic,[10−15] cyclic,[16−19] tadpole-shaped,[20] or star-shaped[16,21−25] pCLs have been prepared with high precision. In particular, the synthesis of end-group modified pCL polymers has raised increased interest due to their use in supramolecular polymer science,[26−30] enabling the attachment of specific end-groups to pCL-based polymers, in turn allowing the generation the formation of self-assembled structures. We[31] and others[32−36] have reported on the attachment of complex hydrogen bonds onto the pCL backbone after a suitable ROP process, which usually allows control of chain length due to its living polymerization behavior. In our methodology, ROP using an alkyne-based initiator is combined with the alkyne/azide "click" reaction,[31,37−42] that allows the attachment of various functional end-groups onto the pCL chain. Based on such modifications, there is a strong quest for analytical tools that enable the identification and characterization of such polymers, in particular revealing their end-groups also those with higher molar masses. The analytical techniques of such pCL-based polymers usually rely on the use of size exclusion chromatography (SEC), which are based on a simple molar mass separation of the polymers.

However, the separation of macromolecules with similar or comparable molar masses having different end-groups often is not feasible. Regarding the case of low molar mass pCLs, NMR spectroscopy allows to monitor the nature of the end-group to the pCL backbone, however, only up to molar masses of ~10000 g·mol^{-1}.

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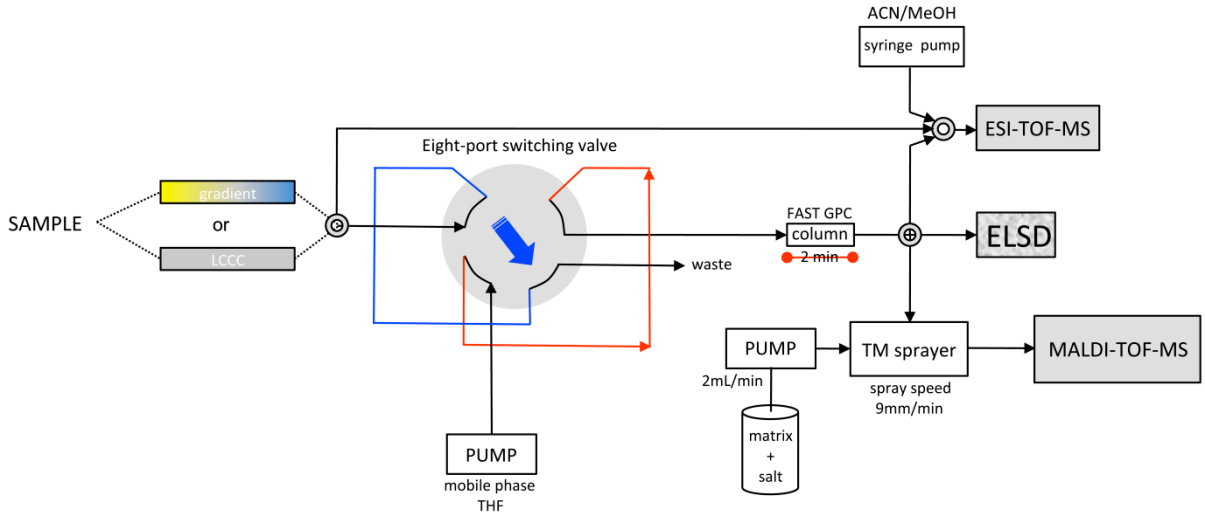
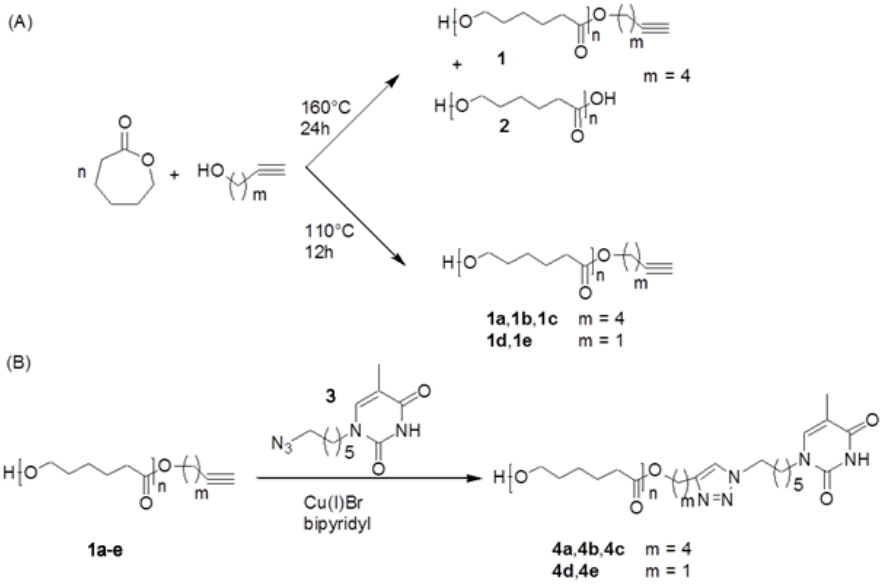
SCHEME 1. Flow chart of the used chromatographic setup (2D-LC/SEC) in combination with mass spectrometric (ESI-TOF or MALDI-TOF) techniques.



SCHEME 2. Synthetic pathways of the end group modification of poly(ε-caprolactones) using ROP and subsequent modification via the azide/alkyne-"click"-reaction.



Additionally, mass spectrometric methods, in particular MALDI-TOF MS[43−47] and ESI-TOF MS methods,[48−52] of pCLs facilitate the determination of the end-group structure, given that a sufficient degree of ionization is possible.[53] Thus, mass spectrometric techniques have led to significant achievements in the low molar mass range of pCL with Mn up to 10 000 g·mol^{-1} in the case of MALDI-TOF MS and Mn up to 5000 g·mol^{-1} for ESI-TOF MS,[54,55] especially for achieving a functionality type distribution (FTD). However, to achieve a separation of the macromolecules prior to desorption or ionization, size exclusion chromatography (SEC) or liquid chromatography (LC) is coupled with soft ionization techniques.[53] In the past decades, the use of two-dimensional chromatographic methods has become a valuable tool for the analysis of polymers, e.g., the combination of LC with SEC.[56−62] In two-dimensional chromatography of polymers (2D),[63] LC is performed at critical conditions (LCCC) where entropic and enthalpic interaction behaviors of the polymer backbone and the stationary phase are compensated, thus focusing entirely on the influence of the end-group within the polyzmer chain. Moreover, 2D methods are a powerful tool for the determination of their purity by separating block copolymers (BCPs) from their homopolymers by determining the critical point of adsorption of one specific building block. In this manner, block copolymers[64−66] containing polyethylene oxides (PEO),[67−71] polystyrenes (PS),[71−74] polymethacrylates (PMA),[72,74] and acrylate polymer[74] as well as star-type polymeric architectures[75−77] have been separated and analyzed. Up to now, the critical adsorption point of poly(ε-caprolactones) could only be determined for low molar mass pCLs (M_n = 1000−5000

g·mol^{-1}) in contrast to higher molar mass pCLs which are insoluble in such described mobile phase systems.[78]

In order to analyze pCL polymers with different end-groups in the high molar mass range, we have developed 2D methods coupled to soft ionization techniques to unveil their properties, such as molar mass and chemical composition focusing on their end-group functionality.[65,78–83] We have assumed that a combination of multidimensional chromatographic methods along with spectrometric techniques can be involved to identify compounds that cannot be addressed by one-dimensional chromatography alone.[70,84,85] The additional separation by molar mass is then achieved via a directly coupled SEC, which then allows the separation of molecules with the same end- group, but different molar mass. In the present paper, we report on the coupling of two-dimensional LC/SEC (2D-LC/SEC) with ESI-TOF and MALDI-TOF MS, in the latter case via a transfer module (TM sprayer). Such a combination should unveil the functionality-type distribution (FTD) via reversed phase C18 column (RP) and/or normal phase (NP = NUC- OH and NUC-NH_2) columns, together with the molar mass distribution (MWD) via a serially connected GPC/SEC column, providing mass accuracy by monitoring online ESI-TOF or MALDI-TOF MS besides identifying the end- groups (see Scheme 1).

Experimental Part

Materials. α,ω-Dihydroxy-telechelic poly(ε-caprolactone) samples, which were used to determine the LCCC conditions with Mn = 14 000 g·mol^{-1} (M_w/M_n = 1.4), 45 000 g·mol^{-1} (M_w/M_n = 1.6), and 90 000 g·mol^{-1} (M_w/M_n = 1.7), were purchased from Sigma-Aldrich, Germany, and used without further purification. Further samples of α,ω-dihydroxy functional pCLs, were synthesized in our laboratories as described elsewhere[19] and hereafter. All solvents used in this study were HPLC-grade (VWR international, Germany) and used without further purification.

*Synthesis. Synthesis of the Low Molar Mass Alkyne-Functionalized pCLs (**1 + 2, 1a, 1b, 1c**).* Low molar mass alkyne-functionalized pCLs (1 + 2, 1a, 1b, 1c) were prepared via coordination insertion ring- opening polymerization (ROP) of ε-caprolactone using 5-hexyn-1-ol as initiator and tin(II) 2-ethylhexanoate (Sn(Oct)2) as catalyst. In the case of sample "1 + 2" ($M_{n,\ SEC}$ = 4500 g·mol^{-1}, M_w/M_n = 1.1) the polymerization was conducted at 160 °C for 24 h, leading to the formation of a mixture consisting of alkyne-functionalized pCLs, as well as pCLs containing a carboxylic end-group (Scheme 2 and Figure 3). In the case of samples 1a ($M_{n,\ SEC}$ = 6000 g·mol−1, M_w/M_n = 1.3), 1b ($M_{n,\ SEC}$ = 15 000 g·mol−1, M_w/M_n = 1.3), and 1c ($M_{n,\ SEC}$ = 30 000 g·mol^{-1}, M_w/M_n = 1.4), we decreased the polymerization temperature to 110 °C and the polymerization time to 3 h for 1a, 6 h for 1b, and 12 h for 1c, resulting in the formation of pure alkyne-functionalized pCLs.[31]

*Synthesis of the High Molar Mass Alkyne-Functionalized pCLs (**1d** and **1e**).* A second batch of higher molar mass pCLs 1d,e, $M_{n,\ SEC}$ = 66 400 and 115 400 g·mol^{-1} (M_w/M_n = 1.4 and 1.8), were prepared according to a different, immortal ROP procedure[86] using a (morpholinophenolate)ZnEt catalyst precursor,[87] propargyl alcohol as co-initiator/chain transfer agent, and 1.0 M toluene solutions of caprolactone (500 and 1000 equiv. vs propargyl alcohol, respectively).

*Synthesis of Thymine-Functionalized pCLs (**4a–4e**) via the Azide/Alkyne "Click" Reaction.* Thymine-functionalized pCLs (**4a– 4e**) were synthesized via the azide/alkyne "click" reaction between alkyne-functionalized pCLs (**1a–1e**) and 1-(6-azidohexyl) thymine (3) (Scheme 2B) using 2,2-bipyridyl as a base and Cu(I)Br as a catalyst. In the case of smaller molar mass pCLs (**1a**, **1b**, **1c**), we used 3 times excess of 1-(6-azidohexyl) thymine (**3**) and conducted the reaction for 24 h as described elsewhere.31 In the case of higher molar mass pCLs (**1d**, **1e**), we modified the reported procedure by increasing the amount of 3 to 5 times excess and conducted the reaction for 72 h. In both cases the products of the "click" reactions were purified via short neutral Al2O3 column to remove Cu(I)Br, precipitated in methanol three times to remove 2,2-bipyridyl, and dried in high vacuum. The samples were then analyzed without any additional separation procedures like column chromatography to determine the real efficiency of the azide/alkyne "click" reactions. All details of the synthetic procedure are reported elsewhere.[31]

Chromatographic Methods. Measurements were performed on a Elite-Chrom-HPLC by *Hitachi VWR* equipped with an autosampler, a highly accurate liquid delivery pump at a semi-micro flow rate (max. 2.4 mL/min), a degasser, a diode array detector (DAD) operating at 190 – 900 nm and a column oven with temperature control (temperature set limit = 0 – 70 °C). The separation of pCL samples was carried out on three different stationary phases. For the gradient LC separation, a reversed phase *Waters* Atlantis C18 column, 100 Å, 5 µm, dimension 4.6 x 250 mm was used (RP-C18). Acetonitrile (ACN) and tetrahydrofuran (THF) were applied as mobile system. For the LCCC separation, two different silica-based stationary phases were used: a *Nucleosil 100-5 OH* (*NUC-OH*) column, 100 Å, 5 µm, dimension 4.6 x 250 mm, and a *Nucleosil 100-5 NH_2* column (*NUC-NH_2*), 100 Å, 5 µm, dimension 4.6 x 250 mm.

For the two-dimensional experiments, an LC separation of pCL samples based on the gradient profile was performed at a flow rate of 0.3 mL/min starting with a high concentration of ACN in the mobile phase, ACN/THF = 80/20. The adsorption effect of ACN was then reduced by increasing the THF content to ACN/THF = 50/50 within 6 min and further reduced to ACN/THF = 30/70 in 10 min. A linear decrease of the THF concentration, ACN/THF = 80/20 was followed within 4 min, using 2 mg/mL of pCL-samples dissolved in ACN/THF = 60/40. The injected sample volume was 30 µL.

For the LCCC/SEC experiments, the critical point of adsorption (CAP) of ε-caprolactone was determined on different silica columns. The critical conditions were found at DCM/THF = 92/8 on NUC-NH_2 and at H_2O/THF =

17.5/82.5 on NUC-OH, at temperatures of 35 °C and 22 °C, respectively. Temperatures were maintained constant (± 0.2 °C) throughout all experiments and the injected sample volume was 30 µL. The DAD signals were recorded on *EZchrom Elite* software version 3.3.2 SP2 with an operating wavelength from 190 – 900 nm at a sampling width of 200 ms to obtain sufficient data points across peaks.

Chromatography in combination with soft ionization techniques. ***2D LC/SEC-ESI-TOF-MS.*** Fractions collected from first dimension (LC) were split up to the ESI-source and to the second dimension (SEC) via a stainless steel Y-shaped microvolume connector (Cat. no. MT1CS6, Valco Instruments, Houston Texas, USA) with a fitting size 1/16" pore 0.25 mm. An adapter from 1/16" to 1/32" was set before the ESI-source to reduce the flow rate. Spectra were recorded in the positive mode with an accelerating voltage of 4.5 kV, a transfer line with 180 °C and a scan range of 50–15,000 *m/z*. The spectra were processed on a *Bruker Daltonics ESI compass 1.3 for micrOTOF* (Data Analysis® 4.0).

2D LC/SEC. Uninterruptedly, the fractions were further conveyed via the Y-shaped microvolume connector into the second dimension using an electronically controlled eight-port switching valve system (EPC8W, *VICI Valco* instruments, Housten Texas, USA), with (2 x 200 µL) loops. The second dimension consisted of an LC-Pump (*Viskotek/Malvern instruments)* and an evaporative light scattering detector (ELSD) 300S *Softa* (SofTA Corporation, *Colorado*, USA) for the detection. Nitrogen was used as carrier gas, the pressure of the nebulizer was set to 5.0 bar and the evaporative temperature was set to 118 °C. For size exclusion separation, a *PSS* PFG high-speed column with the size 50 x 20 mm (Polymer Standard Services, *PSS*, Mainz, Germany), 300 Å and a particle size 5 µm was used. All measurements in the second dimension were performed with HPLC-grade THF as the mobile phase with a flow rate of 5 mL/min for one-dimensional GPC/SEC.

2D LC/SEC-MALDI-TOF-MS. Further investigation of the functionality type distribution (FTD) by means of MALDI-TOF-MS was also achieved. The sample fractions were continuously conveyed to the TM-sprayer (*Leap Technologies*, Carrboro, North Carolina, USA) by splitting the flow subsequent to PFG column with a microvolume cross-connector (Cat. no. MX1CS6, Valco Instruments, Housten Texas, USA), fitting size 1/16". Dithranol (20 mg/mL) was used as MALDI matrix and sodium trifluoroacetate (1 mg/mL) as cationizing reagent. Dithranol and NaTFA were premixed in a 50:1 v/v ratio (matrix:salt) and transferred using an isocratic pump with a flow rate of 2 mL/min. To achieve a uniform and consistent coating, the nozzle height was set to 8 cm, nitrogen was used as a carrier gas with a flow rate of 5 L/min and the fractions were deposited on MALDI target plates with a spray speed of 9 mm/min at 100 °C in a spray linear mode. The spraying process was monitored using LEAP Technologies control software®. The fractions subsequent to the PFG column received from the microvolume cross-connector were also introduced into the ESI source 2D LC/SEC-ESI-TOF.

RESULTS AND DISCUSSION

Our strategy for analyzing end-functionalized pCLs is based on multi dimensional chromatography in combination with soft ionization techniques (2D LC/SEC-MS), taking advantage of functionality-based separation (LCCC) and the separation based on size (SEC). Focus in this study was the separation of the alkyne- and thymine- end-functionalized poly(ε-caprolactones) (1, 2, 1a-e and 4a-e) as demonstrated in scheme 2, using various chromatographic methods coupled with mass spectrometric techniques. Different sets of pCLs were used, varying their polarity, which was provided by their end group structure (alkyne-pCL (1), pCL-OH (2) and thymine-pCL (4)) or via their different molar masses. As reference materials, pCLs prepared via ROP of ε-caprolactone were used, utilizing hexyn-1-ol (*m* = 4) or propargylic alcohol (*m* = 1) as functional initiator as previously described in literature (see Scheme 2).[10, 11]

This polymerization can be conducted in such a mode to generate either a mixture of alkyne- and carboxylic-telechelic pCL (**1, 2**), or pure alkyne-telechelic pCL (**1a-e**) by varying the polymerization temperature. In the latter case, pure alkyne-telechelic pCLs were obtained at lower temperatures, as illustrated in scheme 2A. Molar masses could be adjusted to $M_{n,\,SEC}$ = 6,000, 15,000, 30,000 g·mol^{-1}, using $Sn(Oct)_2$ as catalyst or, in the case of higher molar masses ($M_{n,\,SEC}$ = 66,400 and 115,400 g·mol^{-1}) using a (morpholinophenolate)ZnEt catalyst precursor.[12] Subsequently, an azide/alkyne "click"-reaction of pure alkyne-pCL (**1a-e**) with the thymine azide (**3**) (M_W = 252.15 g·mol^{-1}) was utilized to attach a strongly polar moiety (thymine) onto the pCL backbone resulting in polymers, which differ only to a minor extent from the unmodified ones in their assigned molar masses as determined by SEC methods, using polystyrene standards (in the range from 1,050 to 1,870,000 g·mol^{-1}) as external calibration.[10, 13] Thus, five different molar masses ($M_{n,\,SEC}$ = 6,400, 15,400, 30,700, 66,790, 115,400 g·mol^{-1}) of thymine end-functionalized poly(ε-caprolactones) **4** were investigated in view of the efficiency of the "click"-reaction (see Table 1). It should be noted that the GPC reports the hydrodynamic size of macromolecules. PS and pCL differ strongly in their hydrodynamic properties. Therefore, the molar mass values of pCL samples were corrected by a factor of 0.56, as reported by Save *et al.*,[14] which provides an more accurate molar mass ($M_{n,\,SEC}$) of the final polymers. Functionality type distribution (FTD) was determined by LC, which represents a significant method for the characterization of macromolecules with different chemical composition. However, LC yields more than a single distribution of the molecular characteristics of the polymers (such as molar mass and chemical composition) since both entropic and enthalpic interactions persist in routine LC measurements.

Table 1. Number Average Molar Mass (Mn = g·mol−1) and Polydispersity Index (PDI) of Alkyne- and Thymine-pCLs (1a−1e, 1 + 2, and 4a−4e) Assigned via Size Exclusion Chromatography (SEC) and Nuclear Magnetic Resonance (^{1}H-NMR) for Low Molar Mass pCLs (**1** + **2**, **1a**, **1b**, **4a**, and **4b**).

	molar mass characterization			
entry	pCL	M_n[a] (g·mol^{-1})	M_w/M_n	M_n[b] (g·mol^{-1})
1	1+2	4900	1.1	3978
2	1a	6000	1.3	4206
3	1b	15000	1.3	9799
4	1c	30000	1.4	--[c]
5	1d	66400	1.4	--[c]
6	1e	115000	1.8	--[c]
7	4a	6900	1.2	9457
8	4b	15400	1.4	10964
9	4c	30700	1.3	--[c]
10	4d	66790	1.4	--[c]
11	4e	115000	1.9	--[c]

[a]Molar mass number-average determined by SEC. [b]Molar mass number-average assigned by NMR spectroscopy. [c]Assignments and/or accurate integrations of the end-groups using NMR spectroscopy were not possible due to high molar masses.

LC-Chromatography of pCLs. As known from liquid chromatography (LC) of polymers, there are different modes of separation depending on the interaction parameter *c*, which originates from the structural unit interaction with the stationary phase.[15] In size exclusion chromatography (SEC), the interaction parameter *c* is negative, whereas it is positive for liquid adsorption chromatography (LAC).[16] However for liquid chromatography at critical conditions, often called the "critical adsorption point" (CAP), the interaction parameter is zero, where the chains of a polymer are in a phase transition of a coil between the non-adsorbed state (SEC) and the adsorbed state (LAC), thus leading to separation either with respect *to the size of chains or the chain's polarity*. Figure 1 shows SEC traces of the alkyne-pCLs (**1a-e**) and thymine-pCLs (**4a-e**) indicating no significant shift of the traces of the thymine-functionalized pCLs (**4**) in comparison to the starting materials (**1**), which would unveil the "click"-reaction. Thus, in order to detect the *end group transformations,* liquid chromatography (LC) was investigated for all samples (**1** and **2**), **1a-e** and **4a-e**. Basically, the repetitive units of the pCL-chain as well as the polarity of the end groups will determine the chromatographic behavior of the polymers. Initially, we searched for a critical range (CR) of molar masses (where the retention does not depend on the molar mass),[17-19] a situation, where entropic and enthalpic interactions are balanced, so that the retention time of one species with a specific end group is independent of its molar mass, thus generating sharp peaks in the LC trace. To this endeavor, several solvent mixtures were investigated systematically (see Figure 2).

Gradient polymer elution chromatography of pCLs. Thus, a gradient elution profile was developed to separate the low-molar mass samples of carboxylic- (**2**) and alkyne-telechelic pCLs (**1**) (M_n ≤ 10,000 g·mol^{-1}). In figure 3, samples of functionalized poly(ε-caprolactone), **1** and **2** ($M_{n,\ SEC}$ = 4,500 g·mol^{-1}) were characterized subsequently via LC-ESI-TOF-MS, previously separating the functionalized pCLs on a RP-C18-column. On this column, the decrease of acetonitrile (ACN) concentration from 80/20 to 50/50 (v/v) allowed the separation of the carboxylic- (**2**) and alkyne telechelic pCLs (**1**) by an elution time of less than 1 min at R_t = 10.2 and 10.7 min, respectively. The structure of alkyne-pCL **1** was proven by comparing the measured isotopic pattern with the simulated structure via the Bruker isotopic pattern software. Figure 3 shows the LC chromatogram (figure 3A) and the recorded ESI-TOF-MS spectrum (figure 3B) demonstrating a FTD distribution of the alkyne-pCL **1** with a repeating unit of 57.22 g·mol^{-1}, which refers to doubly charged ions, as the *m/z* values of the isotopic pattern differ by 0.5 g·mol^{-1}, indicating that the individual polymers are desorbed as doubly-charged molecular ions with *z* = 2. Furthermore, the *m/z* spectrum also contains a measured ion species with a monoisotopic mass of *m/z* = 1,965.6379 g·mol^{-1} which agrees well with the simulated one $[(C_6H_{10}O_2)_{33}OC_6H_{10}Na_2]^{2+}$, *m/z* = 1,965.6440 g·mol^{-1} with less than 10 ppm error.

Thymine-pCL **4a** ($M_{n,\ SEC}$ = 6,900 g·mol^{-1}) was measured according to the above-described method. Due to the thymine polar moiety, pCL (**4a**) elutes earlier from the RP C18 column with R_t= 8.80 min as compared to alkyne functionalized pCL **1** and **2** with R_t = 10.2 and 10.8 min (figure 2), respectively. However, the main drawback of this method is the poor solubility of the longer pCL-chains in the gradient elution profile (high molar mass, $M_{n,\ SEC}$ ≥ 35,000 g·mol^{-1}), together with the limiting ionization efficiency of ESI-TOF-MS at higher molar masses (M_W ≤ 15,000 g·mol^{-1}). Therefore, we took advantage of the second dimension in SEC allowing the determination of the molar mass. Even though more complex, the online techniques are preferred due to the ease of automation. Implementation of mass spectrometry techniques via offline (MALDI-TOF-MS) and online (ESI-TOF-MS) procedures would then prevail the FTD and the exact identification of the end group besides the qualitative MWD resolved from the pCL samples (as discussed in section 3.2).

Liquid chromatography at critical conditions (LCCC). In order to allow for an endgroup-sensitive but molecular-weight insensitive separation of the different PCLs, we have also *investigated the critical conditions (LCCC)* for the alkyne (**1**)-, carboxylic- (**2**) telechelic as well as the thymine-telechelic poly(*ε-caprolactones) (**4**)*. This allows a molecular-mass-independent separation of various molar masses of end group modified pCLs based on their chemical composition (thymine end group from the alkyne-pCL polymers). The combination of both methods (LC and SEC) should then lead to a quantitative information about the end group conversion of pCLs, especially providing information about the efficiency of the azide/alkyne-"click" reaction with increasing molar mass, providing the true yield of the thymine telechelic pCLs (**4**).

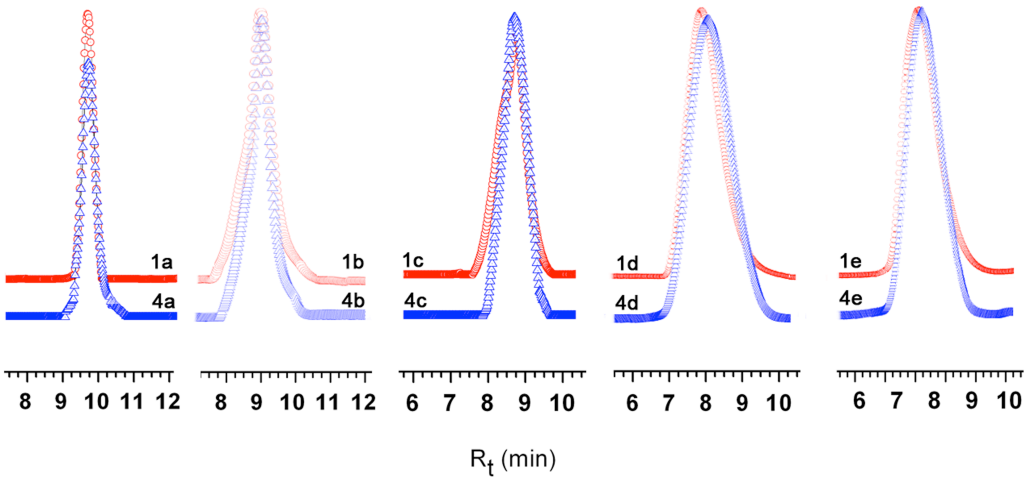


FIGURE 1. SEC traces of alkyne-functionalized (**1a**–**1e**) and thymine-functionalized (4a–4e) pCL.

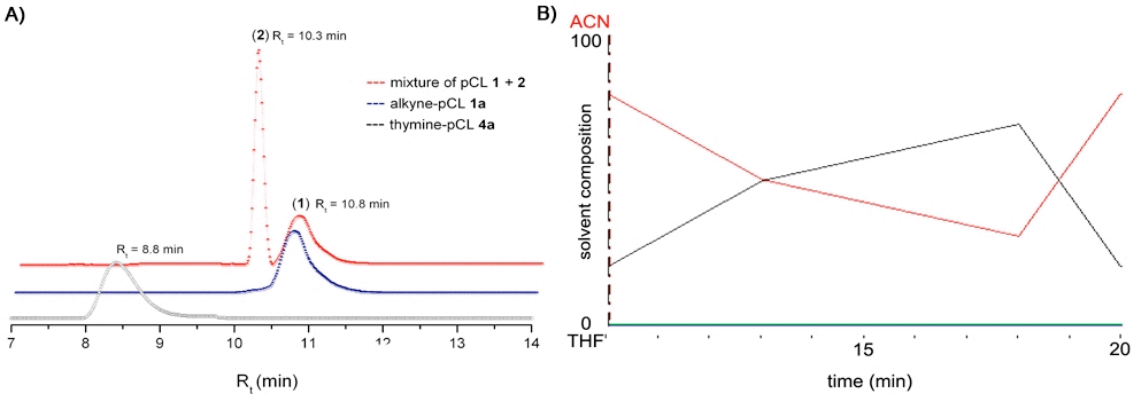


FIGURE 2. (A) LC-UV trace at 220 nm of a ternary mixture of carboxylic- (**2**) and alkyne-pCL (1) ($M_{n,\,SEC}$ = 4900 g·mol^{-1}) (at R_t = 10.2 and 10.7 min) and thymine-pCL **4a** ($M_{n,\,SEC}$ = 6900 g·mol^{-1}) (at R_t = 8.8 min), measured using (B) the shown gradient elution profile. Alkyne-pCL **1a** ($M_{n,\,SEC}$ = 6000 g·mol^{-1}) at R_t = 10.2 min, achieved by ROP at low temperature, T = 110 °C and thymine-pCL **4a** ($M_{n,\,SEC}$ = 6900 g·mol^{-1}) at R_t = 8.8 min, obtained via azide/alkyne "click" reactions.

The practical approach in which a molar mass independent elution was obtained, was achieved on three different silica gel stationary phases, starting with a thermodynamically good solvent such as THF (one component mobile phase). For further investigation, LCCC-conditions of a commercial dihydroxy-telechelic pCL (*Sigma Aldrich*) ($M_{n,\,SEC}$ = 10,000; M_w/M_n = 1.4, 45,000; M_w/M_n = 1.6 and 90,000 g·mol^{-1}; M_w/M_n = 1.6) was probed. Dihydroxy-telechelic pCLs show molar mass-dependent behavior (size-exclusion mode) in THF at 22 °C using the referred stationary columns.

However, by adding up a polar solvent such as H_2O or MeOH to THF, one can observe that the interaction is significantly increased between the stationary phase and the polymer molecules. This interaction is related to the polarity index (P) of the mixed eluent system, which is affected by dispersive forces, dipole-dipole interactions and hydrogen bonding.[20] By mixing H_2O (P = 10.1) or MeOH (P = 5.1) with THF (P = 4.1), the P value of the eluent mixture increases and therefore favors the interaction of the polymer molecules with the eluent, compensating the interaction with the stationary phase. Figure 4 shows the LCCC conditions that have been reached for the pCL homopolymer using a reversed phase (RP-C18) (Figure 4a), a normal phase (*NUC-OH*) with dihydroxy groups (Figure 4b) and an amino-phase (*NUC-NH$_2$*) with attached aminopropyl group (Figure 4c). The poor solubility of the thymine-pCLs in the eluent mixture (Figure 4a) limits the range of detection to 35,000 g·mol^{-1}, together with a low quality in the LAC mode with respect to the end group functionality.

A similar effect was observed in figure 4b. The high molar mass thymine-pCLs (**4**) were detected in two thermodynamically good solvents DCM/THF, $M_{n,\,SEC}$ = 115,400 g·mol^{-1} (Figure 4c). However, the results demonstrated indicate that the separation quality of the dihydroxy-telechelic pCL and the alkyne-modified pCL (**1a-e**) is rather poor due their structural and conformational similarity.

Multi-dimensional chromatography (2D-LC/SEC) of pCLs. *Coupling of gradient LC with SEC monitored by mass spectrometry.* In order to fully profit from the advantages of 2D LC/SEC, mixtures of alkyne- and thymine- telechelic pCLs (see scheme 2, compounds 1, 2, 1b, 1c, 4b and 4c) with defined molar masses, all resulting from ROP using Sn(Oct)2 catalyst at 110 °C (alkyne-pCL) and the subsequent azide/alkyne-"click" reaction (thymine-pCL, **4**) were investigated using the developed gradient-elution technique of LC-chromatography.

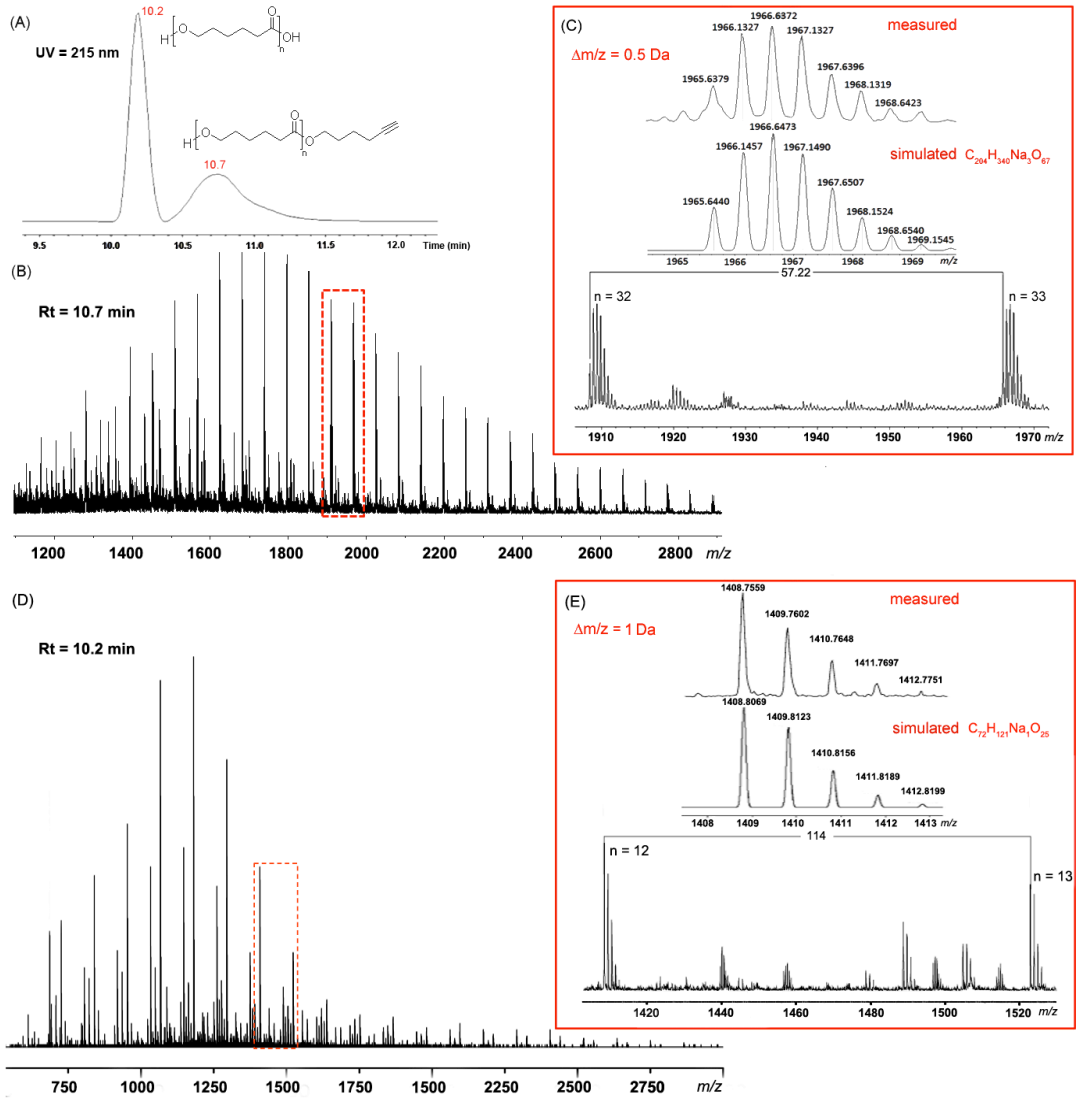


FIGURE 3. (A) LC-ESI-TOF-MS of pCL (**1** and **2**) indicating the separation of carboxylic- and alkyne telechelic poly(ε-caprolactones) via linear gradient profile of ACN and THF. (B, C) showing the comparison between the measured and simulated isotopic pattern of a doubly charged species (alkyne-pCL, R_t = 10.7 min) proving their final structure. (D, E) demonstrates the recorded ESI-TOF-MS spectrum of carboxylic-pCL (R_t = 10.2 min) comparing their measured and simulated isotopic pattern.

The 2D LC/SEC chromatogram (see Figure 5) unveils the separation of both alkyne- and hydroxy- telechelic pCL **1** and **2** shown figure 5a. Premixed polymers **1b** and **4b** in a ratio of 1:1 wt% clearly indicated a complete separation based on end group functionality of both components (same molar masses, M_n = 15,000 g·mol^{-1}). Upon premixing of the samples with different molar mass and different end groups, samples **1b** (15,000 g·mol^{-1}) and **4c** (30,000 g·mol^{-1}) in a ratio of 1:1 wt% a clear separation of both component spots in the 2D-chromatogram could still be observed (Figures 5c and 5d). Premixing of **1a** (low molar mass alkyne-pCL) with **4c** (high molar mass thymine-pCL) in a ratio of 1:1 (wt%), proves the separation of pCLs upon FTD and via the different molar masses. A quantitative determination of the premixed pCL polymers with various molar masses is summarized in Table 2. To identify the chemical structure of the mixture of compounds and further prove their chemical composition, the two-dimensional LC/SEC-setup was coupled online with ESI-TOF MS and offline with MALDI-TOF MS by means of a spray transfer module. Figure 6 shows fractions collected subsequent to the second dimension to ESI-TOF-MS, where compound **4b,** thymine-pCL ($M_{n,\,SEC}$ = 15,000 g·mol^{-1}) is strongly ionized in higher order charge states ($\Delta m/z$ = 0.11, z = 10).

The monoisotopic mass ($\Delta m/z$= 2,448.7922 g·mol^{-1}) in figure 6B agrees with the simulated isotopic pattern ($\Delta m/z$ = 2,448.9385 g·mol^{-1}) of the species $[H(C_6H_{10}O_2)_{82}C_{17}H_{26}O_3N_5Na_4]^{4+}$, as do the deconvoluted masses of the thymine-pCL, M_p = 11,439 g·mol^{-1} and for the alkyne functionalized pCL, M_p = 9,847 g·mol^{-1} (Figure 6). Thus, pCL-polymers up to a molar mass of 10,000 g·mol^{-1} can be separated by 2D LC/SEC and directly detected and thus identified chemically by ESI-TOF-MS. Consequently, the "click"-reaction efficiency was successfully determined in view of the high molar mass pCLs.

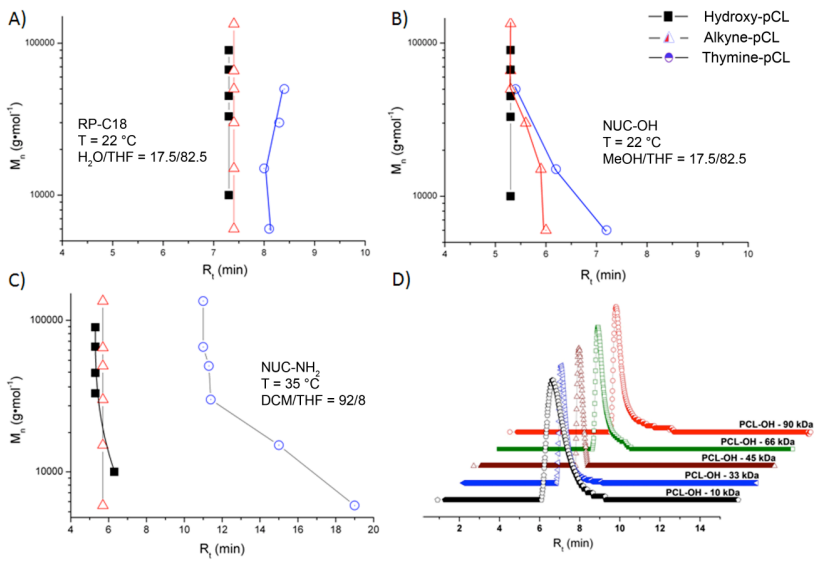


FIGURE 4. Plots of log M_n vs. R_t for hydroxy- (black squares), alkyne- (**1**, red triangles) and thymine-pCL (**4**, blue circles) at defined temperature, solvent compositions and selected stationary phase. *α,ω*-Dihydroxy telechelic poly(*ε*-caprolactone) is eluted under critical conditions at A) using an Atlantis RP C18-column at a temperature of 22 °C and a mobile phase: H_2O/THF = 17.5/82.5; B) a NUC-OH-column at a temperature of 22 °C and a mobile phase: MeOH/THF = 17.5/82.5 and C) a NUC-NH_2- column at a temperature of 35 °C and a mobile phase: DCM/THF = 92/8. D) graphical representation of the LCCC conditions of dihydroxy-pCL (pCL-OH) for the solvent-mixture used in (C).

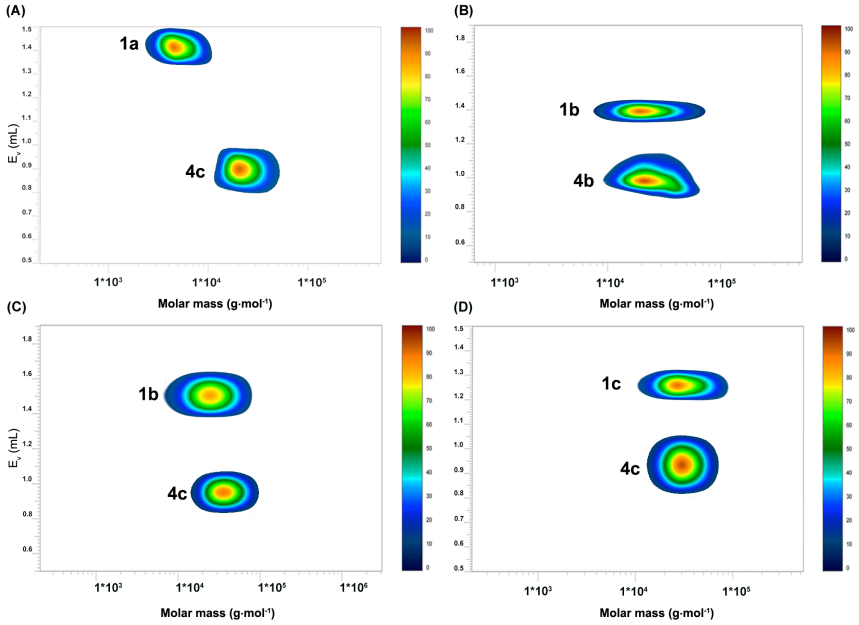


FIGURE 5. 2D LC/SEC of (A) 2D plot of a premix of **1a** and **4c** in a ratio of 1:1 wt% performed in a linear gradient profile of ACN/THF (B) 2D plot of a premix of **1b** and **4b** in a ratio of 1:1 wt% (C) 2D plot of a premix of **1b** and **4c** in a ratio of 1:1 wt%, and (D) 2D plot of a premix of **1c** and **4c** in a ratio of 1:1 wt%. The temperature was kept constant (T = 22 °C) through the total gradient profile with a flow rate of 0.1 mL/min.

To estimate the ionization efficiency of pCLs, a MALDI-TOF mass spectrum acquired by a sample transfer module (SEC-MALDI-TOF-MS) was measured. Both spectra in figure 7 (A1 and A2) could be assigned to thymine- (**4b**) and alkyne-pCL (**1b**), respectively. The molar mass of thymine-pCL (**4b**) (M_p = 9,271 g·mol^{-1}) extracted from figure 7 A1, is significantly lower when compared with the deconvoluted mass observed in figure 6B (M_p = 11,439 g·mol^{-1}). However, the difference is slightly less in alkyne-pCL (**1b**, M_p = 9,017 g·mol^{-1}), compared to the measured mass via ESI-TOF-MS (M_p = 9,847 g·mol^{-1}). Such results clearly show that the ionization efficiency of thymine-pCL in ESI-TOF MS is stronger due to heavily charged ions and less signal interference as compared with MALDI-TOF-MS.

Table 2. Quantification of a 1/1 mixture of the synthesized alkyne-pCLs via ROP and thymine-pCLs prepared via azide/alkyne "click"-reaction using 2D (LC/SEC) liquid chromatography in combination with ESI-TOF-MS and MALDI-TOF-MS.

			Quantification of premixed pCLs					
			2D-LC/GPC		2D LC/SEC-ESI-TOF-MS		2D LC/SEC-MALDI-TOF-MS	
entry	pCL mix	ratio (wt%)	alkyne (mol%)	thymine (mol%)	alkyne (mol%)	thymine (mol%)	alkyne (mol%)	thymine (mol%)
1	1a + 4c	1:1	47.8	52.2	37.2	--[a]	23.7	--[a]
2	1b + 4b	1:1	46.3	53.7	39.4	60.6	27.1	72.9
3	1b + 4c	1:1	47.1	52.9	34.7	--[a]	21.8	--[a]
4	1c + 4c	1:1	48.2	51.8	--[a]	--[a]	--[a]	--[a]
5	1c + 4c	1:1	46.5	53.5	--[a]	--[a]	--[a]	--[a]

[a]High molar mass pCLs (M_n > 15 000 g·mol^{-1}) were not detected in ESI-TOF-MS and MALDI-TOF-MS

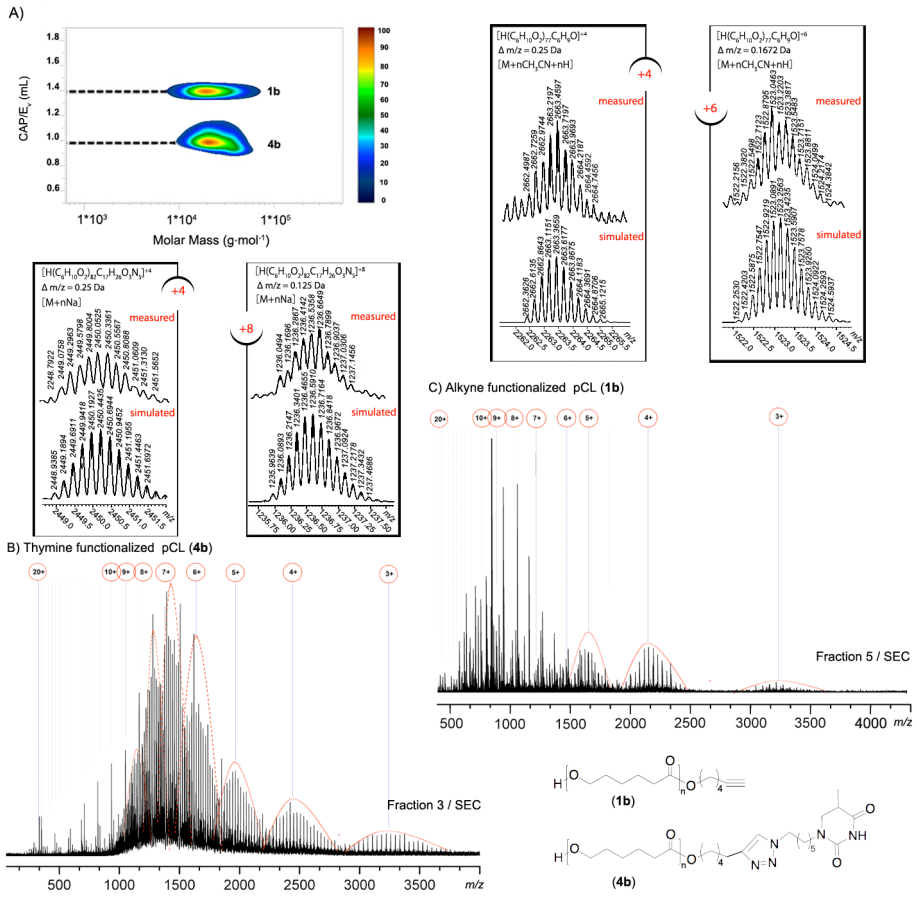


FIGURE 6. 2D LC-SEC-ESI-TOF-MS of a mixture of **1b** and **4b** in a ratio of 1:1 wt% was characterized to identify their functional groups. Fraction number 3 represents one SEC measurement in the two-dimensional 2D LC/SEC experiment, which is automatically conveyed to the mass source via cross connectors. B) Thymine-pCL **4b** and C) alkyne-pCL **1b** were observed via the multiply charged ions (z = 10) in which their simulated structures agree with measured ones. The molar mass distribution represented by M_p = 11,439 g·mol^{-1}.

The MALDI-ionized molecules typically have m/z values comparable with their molar mass (z = 1) besides interference in the signal quality due to the strong ionization/desorption of the matrix molecules, which suppresses ionization of polymer molecules. On the other hand, MALDI- and ESI-TOF-MS signal intensities ratio can be used to quantify the polymer content in polymer mixture.[21] As example, the determination of the alkyne-pCL content **1** in thymine modified pCL **4** after successful synthesis can be investigated, after obtaining a reliable calibration curve based on the chemical composition and molar mass discrimination of a compound. Therefore, pure alkyne- (**1a**; M_n = 6,000 g·mol^{-1} and **1b**; M_n = 15,000 g·mol^{-1}) and thymine-pCLs **4a**; M_n = 6,900 g·mol^{-1} and **4b**; M_n = 15,400 g·mol^{-1}) were mixed with different ratios increasing the amount of thymine-pCL component and investigated by both methods based on their ionization efficiencies. The intensities of individual species based on their degree of polymerization are plotted against the correlated mol%, yielding the individual sensitivity values for the corresponding ions (see Figure S2, supplementary).

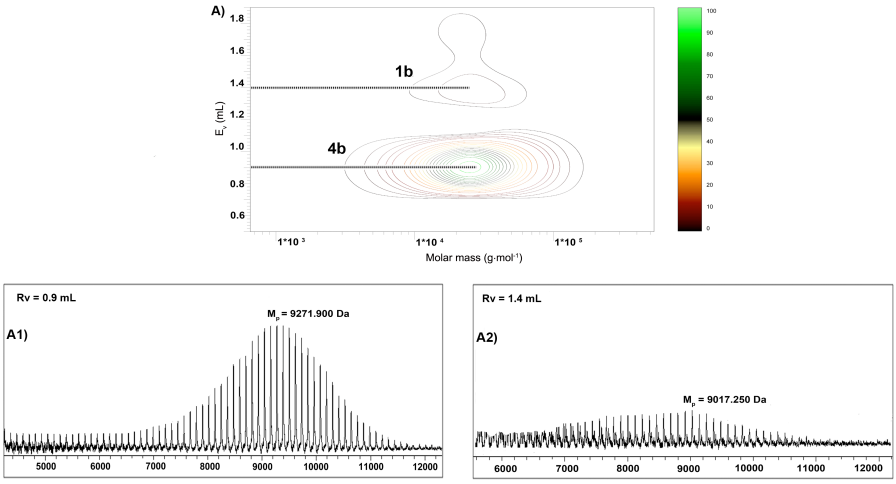


FIGURE 7. 2D LC/SEC-MALDI-TOF-MS of the premixed mixture of **1b** and **4b**, the MALDI spectrum of B) thymine-pCL, **4b**, (M_n, SEC = 15,400 g·mol^{-1} and C) alkyne-pCL, **1b**, (M_n, SEC = 15,000 g·mol^{-1}) which were successfully identified and assigned to their measured structures.

As shown in Table 2, several mixtures of alkyne and thymine-pCLs were therefore investigated using the 2D-LC/SEC coupled with mass spectrometric methods (ESI-TOF-MS and MALDI-TOF-MS). It is significant to notice that alkyne- and thymine-pCL resulted from ESI-TOF-MS calibration curve (**1b** = 39.4 mol% and **4b** = 60.6 mol%) differ from the corresponding MALDI-TOF-MS (**1b** = 27.1 mol% and **4b** = 72.9 mol%) due to their ionization efficiency, which is weaker for alkyne-pCL in MALDI when compared to ESI-TOF MS. Quantification analysis by ESI-TOF-MS revealed significant differences in the quality and efficiency of ionization process once compared to the 2D-LC/SEC.

Coupling LC with SEC utilizing critical conditions for pCLs. To check the efficiency of the azide/alkyne "click"-reactions performed on low and higher molar mass pCLs, 2D LCCC/SEC experiments were carried out for the alkyne- (**1a-e**) and thymine-pCLs (**4a-e**) after conversion. Thus a mobile phase mixture of DCM/THF = 92/8 on a NUC-NH_2 stationary phase was found to display the critical conditions for the hydroxy-pCL and employed to reveal the degree of end group functionalization. Yet, the retention time depends exclusively on the end group functionality, which increases drastically by introducing the thymine moiety onto the pCL backbone (see figure 4c). The 2D LCCC/SEC plots in figure 8 (**A-D**) show the results obtained from the alkyne-pCL (**1a-e**) with increasing molar mass up to 115,400 g·mol^{-1}, with R_v = 1.8 mL (R_t = 18 min). The data for thymine-pCL (**4a-e**) in 2D plots recorded after the azide/alkyne-"click" reaction between alkyne-pCLs and thymine azide are shown in figure 8 (E-H), depicting the conversion efficiency by observing residual spots of the respective alkyne-functionalized starting material. By comparing the volume percentage of the thymine- and alkyne-pCL spots, a quantitative information about the ratio between both compounds is received, which provides an account about the azide/alkyne-"click" efficiency with increasing molar mass. In figure 8E, the thymine-pCL (**4a**) $M_{n,\,SEC}$ = 6,000 g·mol^{-1}, R_v = 5.7 mL, shows a complete conversion, as no residual alkyne-pCL (**1a**) can be detected. By increasing the molar mass ($M_{n,\,SEC}$ = 30,000 g·mol^{-1}, R_v = 3.3 mL), a residual amount of ~3 mol% of alkyne-pCL (**1c**) can still be observed. By further increasing the molar mass up to $M_{n,\,SEC}$ = 115,400 g·mol^{-1}, a residual unreacted amount of 44 mol% of the alkyne-pCL (**1e**) was determined, indicating that the reaction efficiency is significantly reduced by increasing the molar mass (see table 3).

Conclusion

End functionalized poly(ε-caprolactones) varying in their molar mass (ranging from 4900 to 115,000 g mol^{-1}) have been investigated extensively via two-dimensional chromatographic methods (2D-LC/SEC) coupled to soft ionization techniques to unveil their physicochemical properties, such as molar mass, chemical composition and purity. The combination of multi-dimensional chromatographic methods (e.g. LC/SEC) with spectrometric techniques can identify compounds, which cannot be interpreted by one-dimensional chromatography alone. Thus, the present paper demonstrated two different strategies for analyzing end functionalized pCLs up to a molar mass of 115,000 g·mol^{-1}, depending on their solubility in the utilized solvent system. It was found that a combination of LC with SEC using a gradient elution profile was sufficient to separate telechelic pCLs up to 35,000 g·mol^{-1}, demonstrating a useful method, which allows a quantification of the efficiency of the pCL end group modification. However, the poor solubility of high molar mass pCLs (above 35,000 g·mol^{-1}) in the probed gradient solvent system forced us to develop a universal 2D-method (LC/SEC) to separate/identify low and high molar mass pCLs. We found that the separation under critical conditions (LCCC) using an isocratic solvent mixture of DCM/THF (92/8, v/v) was independent of the molar mass, allowing it to separate pCLs based on the end group heterogeneity.

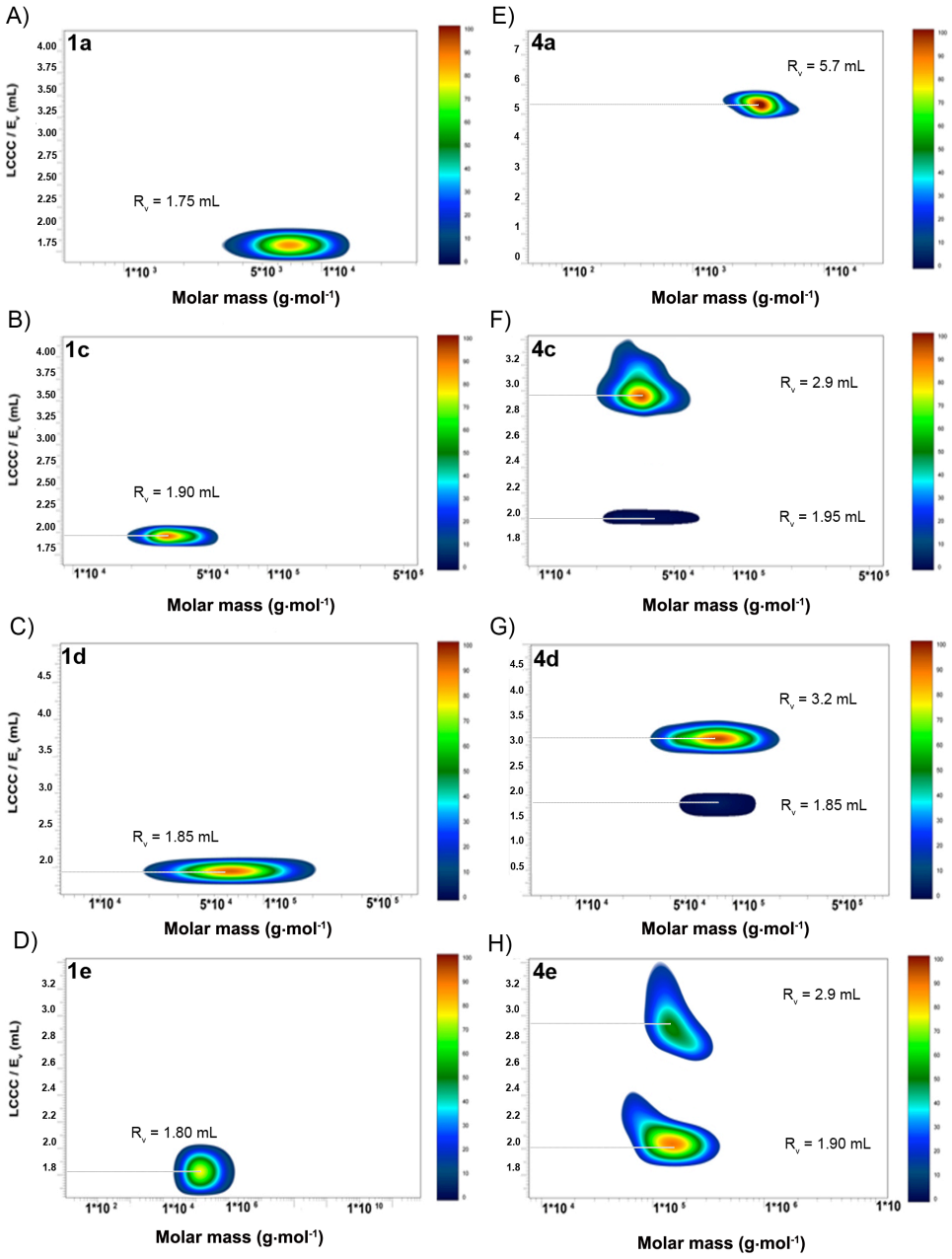


FIGURE 8. LCCC/SEC diagram of alkyne-functionalized pCLs (**1a**-**e**) and the corresponding thymine-functionalized pCL **4a**-**e** prepared via alkyne/azide "click"-chemistry. The efficiency of the "click" reaction reduces with increasing the molar mass of the pCLs.

Table 3. Quantification analysis based on linear direct response (ELSD) of thymine-functionalized pCLs **4a**-**e** subjected to azide/alkyne - "click"-reaction using Cu$^{(I)}$Br as a catalyst and 2,2-bipyridyl as a base.

		quantification of thymine-functionalized pCLs					
		"click"-reaction conditions				2D-LC/SEC[b]	
entry	pCL	amount of 3[a] (equiv.)	solvent	temperature (°C)	time (h)	alkyne (mol%)	thymine (mol%)
1	4a	3			24	0	100
2	4b	3	DCM/THF		24	1	99
3	4c	3	92/8	80	24	3	97
4	4d	5			72	9	91
5	4e	5			72	44	56

[a]The amount of 1-(6 azidohexyl) thymine 3 is given in equivalents (equiv) relative to the alkyne-functionalized pCLs. [b]Yields are provided with an error of ± 0.5 - 2 mol%.

We thus have revealed that the efficiency of the azide/alkyne-"click"-reaction which was used to introduce the thymine functionality on poly(ε-caprolactones) (**4a-e**) is strongly affected by the molar mass of the alkyne-pCLs (**1a-e**). By increasing the molar mass above 66,400 g·mol^{-1}, a residual amount of alkyne-pCL was observed and identified in the final "click"-product. In particular, thymine-pCL of 115,000 g·mol^{-1} (**4e**) has shown a residual amount of 44 mol% of **1e**, indicating that the reaction efficiency is significantly reduced with increasing molar mass.

The combination of chromatographic methods with soft ionization techniques (ESI-TOF-MS and MALDI-TOF-MS) allowed a comparison of the ionization efficiencies observed by the two different spectrometric techniques. Our results clearly show that the ionization efficiency of investigated alkyne- or thymine-pCLs in ESI-TOF-MS is much stronger when compared to MALDI-TOF-MS, which is identified by heavily charged ions and their reduced signal interference. In the contrary, the MALDI ionization method is limited to the detection of singly charged ions from matrix clusters, which strongly suppress signal intensities of the analytes. The method developed herewith offers a convenient mode to analyze the end group nature and end group-distribution of poly(caprolactone) polymers reliably via a relatively simple setup, managing to separate such small changes within the end groups in pCL-polymers.

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Abbreviations

ACN, acetonitrile; **CR**, critical range of molar masses (where retention does not depend on the molar mass); **DAD**, diode array detector; **DCM**, dichloromethane; ELSD, evaporative light scattering detector; **ESI-TOF-MS**, electrospray ionization – time of flight – mass spectrometry; **FTD**, functionality type distribution; **LAC**, liquid adsorption chromatography; **LC**, liquid chromatography; **LCCC**, liquid chromatography at critical conditions; **MALDI-TOF-MS**, matrix assisted laser desorption ionization – time of flight – mass spectrometry; **MWD**, molar weight distribution; **M_n**, number average molar mass; **NP**, normal phase silica gel column; **NUC-NH_2**, Nucleosil 100-5 NH_2; **NUC-OH**, Nucleosil 100-5 OH; **RP-C18**, Atlantis RP-C18; **RI**, refractometric detector; **RP**, C-18 reversed phase column; **SEC**, size exclusion chromatography; **TM sprayer**, transfer module sprayer; **THF**, tetrahydrofuran; **2D**, two-dimensional chromatography; **2D LC/SEC-ESI-TOF-MS**, liquid chromatography coupled in parallel to size exclusion chromatography and **ESI-TOF-MS; 2D LC/SEC**, LC coupled to SEC; **2D LC/SEC-MALDI-TOF-MS**, LC coupled in series to SEC and MALDI-TOF-MS (via TM-sprayer); **2D LC/SEC-ESI-TOF-MS**, LC coupled in series to SEC and ESI-TOF-MS (online).

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SUPPORTING INFORMATION

FIGURES

MALDI-TOF-MS

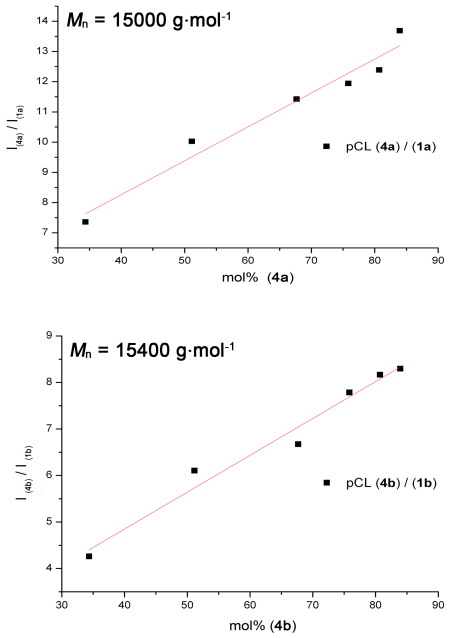

FIGURE S1. ESI-TOF-MS calibration curve prepared by mixing alkyne- and thymine-pCL (1a and 4a) with increasing thymine-pCL content plotted against the intensities of the individual peak intensities of selected ions with a specific degree of polymerization (n).

ESI-TOF-MS

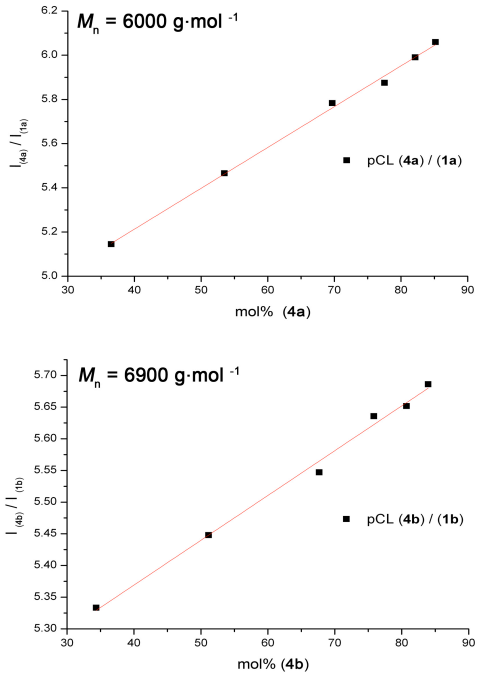

FIGURE S2. MALDI-TOF-MS calibration curve prepared by mixing alkyne- and thymine-pCL (1a and 4a) with increasing thymine-pCL content plotted against the intensities of the individual peak intensities of selected ions with a specific degree of polymerization (n).

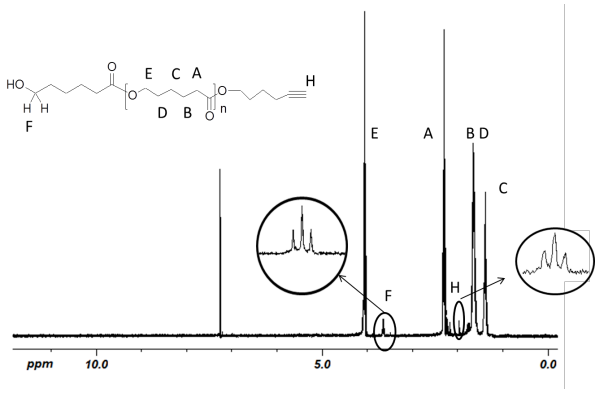

FIGURE S3. ^{1}H NMR spectra of telechelic alkyne-pCL $M_{n\ (NMR)}$ = 4206 g·mol^{-1}resulted via ROP processes.

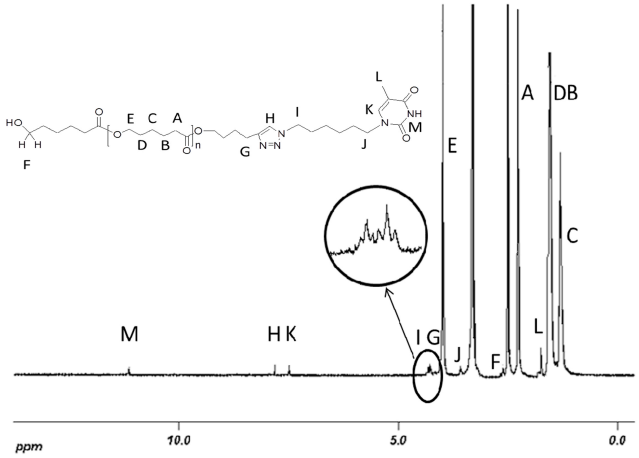

FIGURE S4. ^{1}H NMR spectra of telechelic thymine-pCL $M_{n\ (NMR)}$ = 9457 g·mol^{-1}after end group modification via azide/alkyne-"click"-reaction.

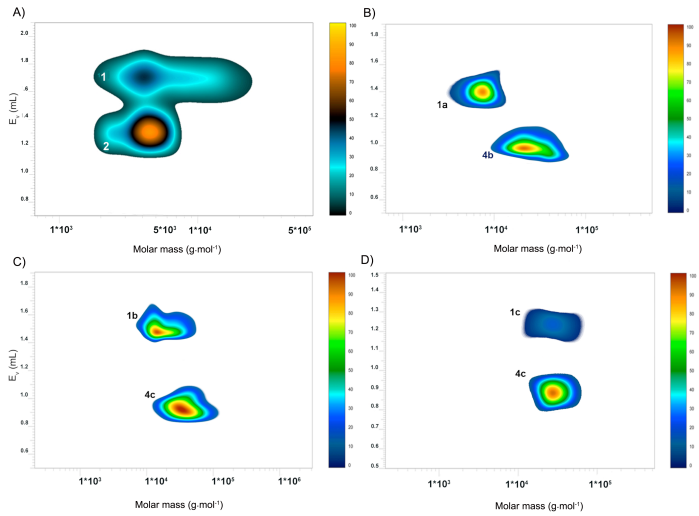

FIGURE S5. 2D LC/SEC performed in a linear gradient profile of ACN/THF (A) 2D plot of a mixture of **1** and **2** (B) 2D plot of a premix of **1b** and **4c** in a ratio of 1:2 wt%, and (D) 2D plot of a premix of **1c** and **4c** in a ratio of 1:4 wt%. The temperature was kept constant (T = 22 °C) through the total gradient profile with a flow rate of 0.1 mL/min.

3.3 2D-LC/SEC-(MALDI-TOF)-MS Characterization of Symmetric and Nonsymmetric Biocompatible PEO_m–PIB–PEO_n Block Copolymers

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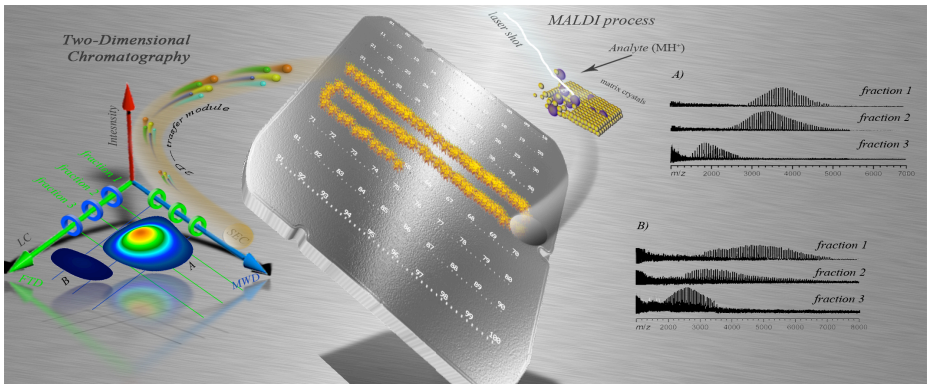

ABSTRACT: Complex copolymer mixtures can be directly analyzed via multidimensional chromatographic techniques after successful synthesis. High-performance liquid chromatography coupled to size exclusion chromatography (LC/SEC) revealed detailed information on the chemical composition, polymeric structure, and molar mass distribution of copolymer mixtures, in particular of symmetric and nonsymmetric α-TEO-ω-PEO telechelic PIB copolymers. A series of azide/alkyne "click" reactions after living polymerization reactions were used to prepare the either symmetric or nonsymmetric PIB–PEO-based triblock copolymers of the general structure (PEO_n–PIB–PEO_m BCPs (with n = 3; m = 3, 12, or 17)). In order to demonstrate the efficiency of the "click" reaction and thus the purity of the final triblock copolymers, the critical conditions of the PIB-homopolymers (M_n = 3–30 kg mol^{-1}) in the isocratic elution mode (LCCC) were investigated. Thus, it was possible to separate the final polymers from their intermediates using a reversed-phase Atlantis-RP C18 column as stationary phase and a mixture of methyl-tert-butyl ether/methanol (85.34/14.66 (w/w)) as mobile phase. On the basis of the PEO segment length and overall hydrophobicity of the BCPs, we observed a complete separation of the stepwise "click" products. Finally, direct coupling of the 2D-LC/SEC to (MALDI-TOF) mass spectrometric techniques allowed a clear identification of all reaction steps proving the structure of the final symmetric and nonsymmetric triblock copolymers.

Introduction

Biomaterials, most of all those based on poly(ethylene oxide),[1-3] represent an enormously important class of polymers in pharmaceutical applications as they can selectively control the release and circulation of drugs via different principles such as diffusion,[4] water penetration,[5] chemically controlled delivery[5] and response.[6] Therefore, changing the chemistry of the polymeric materials, e.g. introducing polyanhydrides,[7] polyesters,[8] polyacrylic acids,[9] poly(methyl methacrylate)[10] and polyurethanes,[11] can enable a selective release of the active agent/drug by shielding the carrier sterically via closely attached PEO-brushes from early cellular recognition and thus renal clearance.[3, 12] This involves hydrophilic, amorphous and low molecular weight polymers carrying functional side groups, in particular, biodegradable and biocompatible block copolymers based on their diversity of recent pharmaceutical applications.[13-15] Of particular interest are amphiphilic BCPs[16] as they can self-assemble into polymersomes[17-26] with a defined membrane thickness, enabling to address drug-delivery systems similar to conventional liposomes, but with significantly higher stability and drug loading.[27-35] As the complexity of macromolecules (*e.g.* block copolymers) increases by the plethora of synthetic routes, more specific and sophisticated analytical methods are required to prove the structure, most of all with respect to their architecture, the attached endgroups, as well as the exact chemical composition. Separation techniques based on the hydrodynamic volume of the analyte (*e.g.* Size Exclusion Chromatography[36] (SEC)) which differ significantly from other separation techniques subjected to chemical interactions, would drive the specific analysis (chemical structure and architecture) of such polymers into difficulties. Therefore, a considerable number of chromatographic techniques have been developed,[37] which enables to address the generation of a profile including the chemical composition distribution (CCD), the nature of functional side chains together with the molar mass distribution (MMD), the microstructure and the underlying polymeric architecture. Many of these aspects

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are based on the exploitation of distinct adsorption effects, which are normally excluded in the SEC, but required in liquid adsorption chromatography (LAC). The discovery of liquid chromatography of synthetic polymers under critical conditions (LCCC)[38-41] benefits nowadays the separation of block copolymers from their homopolymer-precursors based solely on their different functionalities by eliminating the effects of their molecular weight.[42-50] In liquid chromatography, the main separation modes are governed by the interaction parameter *c*, which describes the interaction of the single (polymer) chain with the stationary phase.[51] Whereas, in size exclusion chromatography (SEC) molecules are excluded from the pores in consequence of their hydrodynamic volume (being driven by entropy changes), chromatography in the adsorption mode (LAC) is driven by enthalpic changes of the structural units with the stationary phase, whose distribution coefficient is much larger than one, K > one (see equation **1**).

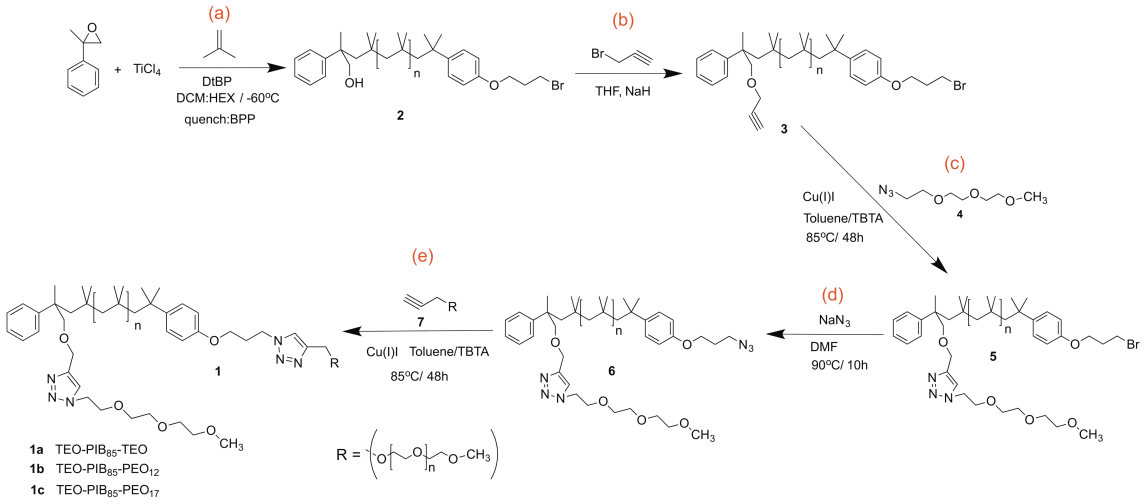
$$ln\ K = \Delta G = \Delta H - T\Delta S \qquad (1)$$

The critical adsorption point (CAP), where all structural units of the polymer coelute together with the initial solvent regardless of their chain length is given by the compensation of enthalpic and entropic driving forces. Therefore, putting the distribution coefficient K = one, as well as the interaction parameter c = zero,[52-55] and is usually found by a careful match among solvent-composition, the respective polymer and the polarity of the used separation matrix. In the analysis of di- and triblock copolymers (A_nB_m or $A_nB_mA_n$ types), the chromatographic separation method is based on the critical condition of the homopolymer *A* (LCCC) and hence, leading to an elution of a block copolymer - depending on the exact nature of its interaction with the stationary phase – either in the exclusion - (SEC) or the adsorption mode (LAC). Starting from the well-known LCCC conditions of PEO[56]- and PMMA[57]-based polymers, a large variety of different A_nB_m block copolymers (*e.g.* poly(ethylene oxide)-*b*-poly *propylene* oxide,[58] polystyrene-*b*-poly(ethylene oxide)[59] and polystyrene-*b*-poly(methyl methacrylate),[60] has been analyzed using either one- or two-dimensional chromatography,[47, 48, 61-64] where the block composition varies strongly in their polarities.[65] Especially, the direct coupling of one- and two-dimensional chromatographic methods to mass spectrometry (MALDI and ESI)[66-71] has enabled to effectively combine the separation of BCPs based on functionality and hydrodynamic volume (SEC) into an online two-dimensional system, where an orthogonality criterion is fulfilled via distinctive separation mechanisms.[72] Thus, fractions collected and separated in the second dimension (SEC) can be directly split into MALDI-TOF MS via a sample transfer module or ESI-TOF MS by direct online coupling, where the mass spectra are recorded and correlated with two-dimensional outcome fractions.[68] In this article, we would like to highlight the multi-dimensional chromatographic analysis of either symmetric triblock copolymers (A_nBA_n) tri(ethylene oxide)-*b*-polyisobutylene-*b*-tri(ethylene oxide) (TEO-PIB-TEO (**1a**)) or nonsymmetric (A_nBA_m) poly(ethylene oxide)-*b*-polyisobutylene-*b*-tri(ethylene oxide); PEO_m-PIB-PEO_n (**1b**, **1c**). These and structurally-related block copolymers can form either block copolymer micelles or polymersomal membranes[73] in dependence of their PIB/PEO ratio, which in turn is controlled via living polymerization methods. Both blocks (the PIB and the PEO-block) are biocompatible polymers, thus being prospective polymers for drug-delivery purposes. The applied synthetic methodology follows a combination of living carbocationic polymerization (LCCP) coupled to "click"-chemistry[74-77] as an efficient tool to covalently link two different polymer chains. The use of the initiator methylstyrene epoxide in the LCCP of isobutylene specifically enables to prepare block copolymers with either a symmetric or nonsymmetric composition.[78] The stepwise "click"-reaction has been monitored using 2D-LCCC/SEC coupled to MS (MALDI-TOF MS) to follow the reaction pathways and analyze the exact chemical structure. The purpose of this article is to demonstrate the distinctive role of multi-dimensional chromatographic methods coupled to mass spectrometric techniques to analyze the purity and the structural arrangement of symmetric or nonsymmetric BCPs.

EXPERIMENTAL PART

Materials. All chemicals were purchased from Sigma-Aldrich (*Schnelldorf*, Germany) and were used as received unless otherwise stated. All solvents, which were used for the workup procedures, were distilled prior to use. Tetrahydrofuran (THF) and toluene were pre-dried over potassium hydroxide for several days, refluxed over sodium/benzophenone under an argon atmosphere and distilled. Methylstyrene epoxide (MSE) (initiator was synthesized by the epoxidation reaction of methyl styrene with pure 3-chloro-1-peroxybenzoic acid (MCPBA) in dichloromethane (CH_2Cl_2) according to reference.[79] α-methoxy-ω-azido-telechelic triethylene oxide (**4**) was prepared in a two step reaction starting with the mesylation of the hydroxyl endgroup followed by conversion into the azido-telechelic compound via nucleophilic substitution using sodium azide (see supplementary), as reported in Literature.[80] The preparation of α-methoxy-ω-alkyne-telechelic triethylene oxide (**7**) or α-methoxy-ω-alkyne-telechelic poly(ethylene oxides) (**8**, **9**) was performed via phase transfer catalysis (see procedure, supplementary) according to a representative procedure by Teodorescu et al.[81, 82] Bisallyl-telechelic polyisobutylenes, which were used to determine the LCCC conditions with M_n = 3000 (M_w/M_n = 1.2), 9000 (M_w/M_n = 1.1) and 29 300 (M_w/M_n = 1.2) g·mol^{-1} were synthesized via a living carbocationic polymerization (LCCP) method using the bivalent initiator 1-*tert*-butyl-3,5-bis-(1-chloro-1-methyl-ethyl) benzene (DCCl).[83]

Synthesis. *Synthesis of α-TEO-ω-azido-telechelic PIBs (**6**).* According to literature[84] the synthesis of α-TEO-ω-azido-telechelic PIBs (**6**) was conducted in the same way starting with the polymerization of isobutylene using MSE/$TiCl_4$ as initiator system followed by a stepwise endgroup modification as shown in the supplementary (procedures of all steps). The obtained product was dried



SCHEME 1. Synthetic Pathway toward α-TEO-ω-PEO Telechelic PIBs Using Double "Click" Reactions via Cu(I) Toluene/TBTA at 85 °C.

under high vacuum yielding 87% of (6). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.47 (s, 1H), 6.82 (d, J = 8.41 Hz, 2H), 4.64-4.46 (m, 2H), 4.08 (t, J = 5.78 Hz, 2H), 3.86 (t, J = 4.76 Hz, 1H), 3.72-3.47 (m, 12H), 3.37 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 154.4 (C18), 158.8, 145.4, 142.8, 128.8, 125.7, 125.1, 120.9, 114.2, 81.3, 71.8, 70.6, 69.8, 64.8, 63.5, 59.3, 52.9, 44.3, 43.1, 29.3, 27.6. FT-IR (KBr): ν (cm^{-1}) = 3000-2700 (C-H), ~2100 (N_3).

*Synthesis of symmetrical or nonsymmetrical α-TEO-ω-PEO-telechelic PIBs (**1**).* The azide/alkyne "click"-reaction between α-TEO-ω-azido-telechelic PIB (7) and α-methoxy-ω-alkyne-telechelic poly(ethylene oxides) (8, 9) varying in the number of repeating units was conducted under Cu^I mediated conditions. Compound (6) (1 equiv.), alkyne-telechelic triethylene oxide or poly(ethylene oxide) (1.1 equiv.), (TBTA) (0.1 equiv.) and Cu(I) iodide were dissolved in toluene. The reaction mixture was bubbled with argon for 1 h and then heated up to 80 °C. After 48 hours, the solvent was removed via rotary evaporation and the crude product was purified by column chromatography on silica gel ($CHCl_3/CH_3OH$ = 30:1, R_f = 0.1 to remove the unreacted alkyne-telechelic PEO) yielding symmetrical or nonsymmetrical α-TEO-ω-PEO-telechelic PIB (1). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.59 (s, 1H), 7.47 (s, 1H), 6.82 (d, *J* = 8.41 Hz, 2H), 4.64-4.45 (m, 8H), 4.01 (t, *J* = 5.78 Hz, 2H), 3.86 (t, *J* = 4.76 Hz, 2H), 3.72-3.46 (m, (4m+12)H), 3.37 (s, 6H), 2.38 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 154.4, 158.8, 145.4, 142.8, 128.8, 125.7, 125.1, 121, 114.2, 81.3, 71.8, 69.8, 64.8, 63.5, 59.7, 52.9, 48.3, 43.1, 29.3, 27.8.

Spectroscopic Methods. *Nuclear Magnetic Resonance (NMR) spectroscopy.* ^{1}H- and ^{13}C-NMR spectra were performed on a *Varian Gemini 2000* (400 MHz) FTNMR spectrometer using *MestRec-C* (4.9.9.6) software for data interpretation. The measurements were done in deuterated chloroform ($CDCl_3$: 7.26 ppm (1H) and 77.0 ppm (^{13}C)). All chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethyl silane (TMS); coupling constants (*J*) are given in Hertz (Hz) using standard abbreviations (s = singlet; d = doublet; t = triplet; m = multiplet).

Infrared (IR) Spectroscopy. IR-spectra were recorded on *Bruker Vertex 70* FT-IR spectrometer and evaluated by *OPUS* (6.5) software. Samples were measured with *golden gate unit* combined with a *RT DLa TGS* detector.

Chromatographic methods. *Size Exclusion Chromatography (SEC).* SEC experiments were performed on a *Viscotek GPCmax VE2001* system combined with a *Viscotek TDA30L* (triple detector array). Polyisobutylene standards in the range of 340- 87 600 g·mol^{-1} purchased from PSS (Polymer standard service) were used for conventional external calibration using a *Viscotek VE3580* refractive index detector. The polystyrene divinylbenzene based column set consists of a HHR – HGuard – 17369 pre-column followed by a GMHHR – N – Mixed Bed 18055 (1000 - 4 x 105 g·mol^{-1}) and a G2500HHR - 17354 (100 – 2 x 104 g·mol^{-1}) column. The detector and the column temperature were held constant at 35 °C with a flow rate of 1 mL/min. The investigated samples were dissolved in THF (>99.9%), analyses of the results of the GPC experiments were achieved using *OmniSec* (4.5.6) software®.

High Performance Liquid Chromatography (HPLC). Measurements were performed on a LaChrom Elite® by *Hitachi VWR* equipped with an autosampler, a highly accurate liquid delivery pump at a semi-micro flow rate (max. 2.4 mL/min), a degasser, a diode array detector (DAD) operating at 190 – 900 nm and a column oven with temperature control (temperature set limit = 0 - 70 °C). The separation of the block copolymers, were carried out on a reversed phase *Waters Atlantis-RP C18* column, 100 Å, 5 µm, dimension 4.6 x 250 mm. Methyl-*tert*-butylether (MTBE) and tetrahydrofuran (THF) were applied as the mobile phase system.

Table 1. Characterization of the final triblock copolymers **1a**-**1d** and their precursors **2c**, **3**, **5** and **6** using size exclusion chromatography (SEC), Nuclear Magnetic Resonance (NMR) and their LC retention times with different flow rates (0.1 and 0.3 mL/min).

		characterization						
entry	sample	M_n[a] (GPC)	M_w[a] (GPC)	PDI	M_n[a] (NMR)	chain length[b]	R_t1[c]/ R_t2[d]	Yield[f] [%]
1	HO-PIB85-Br (2c)	4760	5980	1.25	5080	PIB85	---[e]	95
2	Alkyne-PIB85-Br (3)	4920	6200	1.26	5160	PIB85	7.13/31.0	90
3	TEO-PIB85-Br (5)	4840	6100	1.26	5360	TEO-PIB85	---[e]	82
4	TEO-PIB85-N3 (6)	4780	5920	1.24	5300	TEO-PIB85	6.71/29.4	87
5	TEO-PIB85-TEO (1a)	5280	6490	1.23	5500	TEO-PIB85-TEO	6.33/27.1	78
6	TEO-PIB85-PEO (1b)	5030	6240	1.24	5930	TEO-PIB85-PEO12	6.31/27.0	82
7	TEO-PIB85-PEO (1c)	4540	5720	1.26	6150	TEO-PIB85-PEO17	6.31/27.0	67
8	TEO-PIB57-TEO (1d)	4080	5220	1.28	3960	TEO-PIB57-TEO	6.35/27.1	80

[a]Number or weight average molar mass of the BCPs in g·mol^{-1}. [b]Calculated number of the single polymer building blocks by NMR-integration. [c]HPLC retention time (R_t1) of the investigated samples with 0.3 mL/min flow rate. [d]HPLC retention time (R_t2) of the investigated samples with 0.1 mL/min flow rate used in the 2D-HPLC/SEC setup. [e]Sample was not measured via HPLC or 2D-LC/SEC. [f]Isolated mass after precipitation and purification via silica-gel column-chromatography.

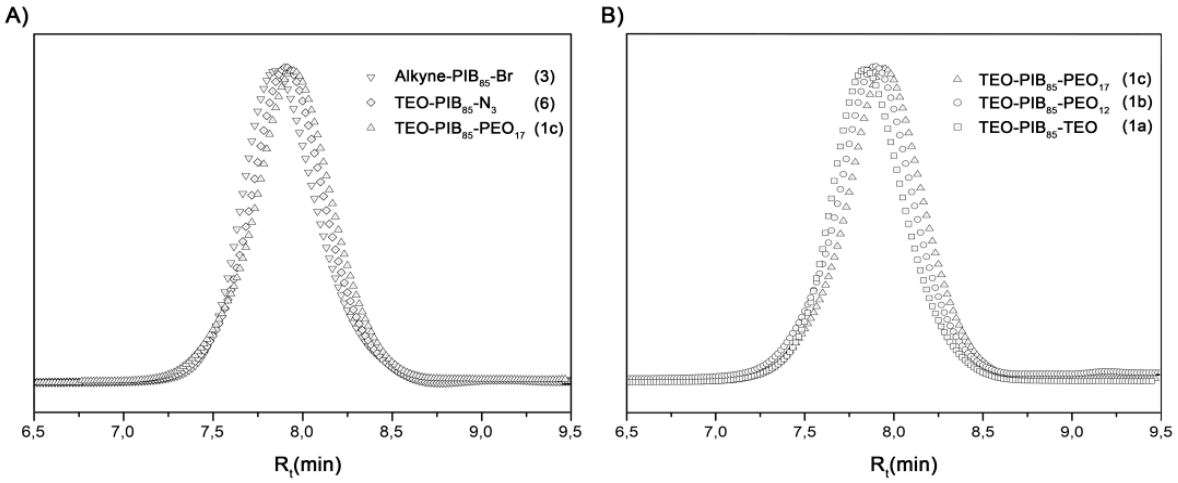


FIGURE 1. SEC traces of (A) the α-alkyne-ω-bromo-telechelic PIB (**3**), the α-TEO-ω-azido-telechelic PIB (**6**) obtained after the 1st-"click"-reaction and (B) the final triblock copolymers (**1a**-**c**) after the 2nd "click"-reaction (see also results in **Table 1**).

The critical condition of bisallyl-telechelic polyisobutylene which further applied to LC/SEC experiments of the block copolymers was found at MTBE/MeOH = 14.66/85.34 *(w/w)* on *RP-C18*, at temperature of 30 °C. Temperatures were maintained constant (± 0.2 °C) through all experiments and the injected sample volume was 20 µL. The DAD signals were recorded on *EZchrom* elite software version 3.3.2 SP2 with an operating wavelength from 190 – 900 nm at a sampling width of 800 ms to obtain sufficient data points across peaks.

Spectrometric methods. *MALDI-TOF Mass spectrometry.* MALDI-TOF MS measurements were performed on a *Bruker Autoflex III* system *(Bruker Daltonics)* operating in reflectron and linear modes. Data evaluation was carried out on *flexAnalysis* software (3.4). Ions were formed by laser desorption (smart beam laser at 355 nm, 532 nm, 808 nm and 1064 nm ± 5 nm; 3 ns pulse width; up to 2500 Hz repetition rate), accelerated by voltage of 20 kV and detected as positive ions. The matrix solution was prepared by dissolving 1,8,9-anthracenetriol (dithranol) or *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) in THF at a concentration of 20 mg/ml. Polymer samples were dissolved in THF at a concentration of 20 mg/ml. AgTFA and LiTFA were dissolved at a concentration of 10 mg/ml in THF. The solutions of the matrix, the polymer and the salt were mixed in a volume ratio of 100:50:4 and 1 µl of each mixture was spotted on the MALDI-target. The instrument was externally calibrated with a 25 poly(ethylene glycol) (PEG) standard (*Mp* = 2000 or 4000 g·mol^{-1}) applying a quadratic calibration method with an error of 1-2 ppm.

Coupling techniques. *Two-Dimensional Chromatography (LC/SEC).* The outlet of the HPLC (first dimension) is connected to the second dimension using an electronically controlled eight-port switching valve system (EPC8W, *VICI Valco* instruments, Housten Texas, USA), with (2 x 200 µL) loops. The second dimension consisted of an LC-Pump (*Viskotek/Malvern instruments)* and an evaporative light scattering detector (ELSD) 300S *Softa* (SofTA Corporation, *Colorado*, USA) for the detection. Nitrogen was used as carrier gas, the pressure of the nebulizer was set to 5.0 bar and the evaporative temperature was set to 118 °C. For size exclusion separation, a *PSS* PFG high-

speed column with the size 50 x 20 mm (Polymer Standard Services, *PSS*, Mainz, Germany), 300 Å and a particle size 5 µm was used. All measurements in the second dimension were performed using HPLC-grade THF as the mobile phase running at a flow rate of 5 mL/min for one-dimensional GPC/SEC. Data acquisition was performed using PSS UDC 810 Universal Data Center, evaluation and extraction of data were accomplished using PSS WinGPC Software®.

Two-Dimensional Chromatography (LC/SEC) coupled to MALDI-TOF MS. Further investigation of functionality type distribution (FTD) by means of MALDI-TOF MS was also achieved. The sample fractions were continuously conveyed to the TM-sprayer (*Leap Technologies*, Carrboro, North Carolina, USA) by splitting the flow subsequent to PFG column with a microvolume cross-connector (Cat. no. MX1CS6, Valco Instruments, Housten Texas, USA), fitting size 1/16". DCTB (20 mg/mL) was used as MALDI matrix and sodium triflouroacetate (1 mg/mL) as cationizing reagent. DCTB and NaTFA were premixed in a 50:1 v/v ratio (matrix:salt) and transferred using an isocratic pump with a flow rate of 2 mL/min. To achieve a uniform and consistent coating, the nozzle height was set to 8 cm, nitrogen was used as a carrier gas with a flow rate of 5 L/min and the fractions were deposited on MALDI-target with a spray speed of 9 mm/min at 130 °C in a spray linear mode. The spraying process was monitored using LEAP Technologies control software®.

RESULTS AND DISCUSSION

Synthesis of symmetric and nonsymmetric tri-BCPs. The synthetic route towards the stepwise construction of the symmetric (α-TEO-ω-TEO block copolymers **1a**) and the nonsymmetric α-TEO-ω-PEO block copolymers **1b** and **1c** is based on methods that have been developed earlier in our group.[84] Using methylstyrene epoxide as initiator for the living cationic polymerization (see **Table S1**, Supplementary) followed by azide/alkyne-"click"-chemistry,[85] thus, enabling the functionalization of the α,ω-endgroup of the PIBs with different moieties (see NMR characterization, Supplementary), as illustrated in **Scheme 1**. Basically, the residual hydroxy-moiety of the initial initiator-moiety allows for an orthogonal functionalization of both polymer chain ends of the intermediate (**2**), as the transformation to the alkyne-PIB_{85}-Br (**3**) now allows the separate attachment of PEO-chains of different chain lengths. The final tri-BCPs **1a**, **1b** and **1c** were thereby synthesized via successive stepwise "click"-chemistry by attaching first a polar triethylene glycol (TEO) unit on the α-endgroup followed by a TEO- or PEO- unit onto the ω-endgroup in high yields with complete endgroup efficiency as judged by excessive NMR spectroscopy (see Supplementary Information). MALDI-investigations and GPC are shown in entry 5, 6 and 7 of **Table 1**. The purpose of this article is to demonstrate the distinctive role of multi-dimensional chromatographic methods coupled to mass spectrometric techniques to analyze the purity and the structural arrangement of the final BCPs together with their synthetic pre-steps.

One-dimensional liquid chromatography (SEC and HPLC). The polyisobutylenes bearing the alkyne endgroup (alkyne-PIB_{85}-Br (**3**)) and the products with the attached hydrophilic moieties, TEO-PIB_{85}-N_3 (**6**) (as diblock copolymer) and TEO-PIB_{85}-PEO_n (**1b**, **1c**) as triblock copolymers were examined using conventional size exclusion chromatography (calibrated against PIB-standards). In **Figure 1**, the GPC-traces of TEO-PIB_{85}-N_3 (**6**), as well as the alkyne-functionalized polyisobutylene precursor α-alkyne-ω-bromo-telechelic PIB (**3**) and the final triblock copolymers (**1a**-**c**) are displayed.

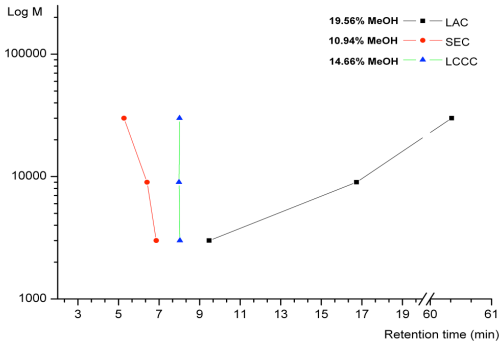


FIGURE 2. Plot of log M_n *vs.* retention time (R_t) for the polyisobutylenes PIB-homopolymer (bisallyl-telechelic polyisobutylenes (M_n = 3 to 30 kg·mol^{-1})), which demonstrate their retention behavior at constant temperature (30°C) using a reversed-phase column (*Atlantis-RP C18*) as stationary phase. By varying the composition of the mobile phase (MTBE/MeOH) different chromatographic interaction modes of the polyisobutylene samples were obtained.

As expected, the peak maximum of the triblock copolymer (**1c**) is slightly shifted towards higher retention volume (TEO-PIB_{85}-PEO_{17} (**1c**), R_t = 7.96 min) compared to the retention volume of the diblock copolymer (TEO-PIB_{85}-N_3 (**6**), R_t = 7.92 min) and the alkyne-functionalized polyisobutylene (alkyne-PIB_{85}-Br (**3**), R_t = 7.84 min), see **Figure 1A**. However, a small shift in the opposite direction can be distinguished by varying the chain-length of the PEO-block in the triblock copolymers **1a**-**c**, as illustrated in **Figure 1B** (TEO-PIB_{85}-TEO (**1a**), R_t = 7.83 min) and (TEO-PIB_{85}-PEO_{12} (**1b**), R_t = 7.91 min). The increased shift in the peak maxima displays a significant change towards lower molar mass values M_n, although the molecular weight is increasing. Experimental GPC results of a series of poly(ethylene oxides) samples varying their chain lengths and architecture showed that longer PEO-chains may lead to reduced hydrodynamic radii due to their limited solubility in THF.[86] Consequently, with increasing the PEO block length in tri-BCPs (**1a** to **1c**), the retention time of the BCPs on the SEC column increases caused by the fact that THF is a poor solvent for the attached PEO blocks resulting in a more contracted chain confirmation (lower hydrodynamic radius). The practical approach in which LCCC is projected for polyisobutylene starts with a thermodynamically good solvent such as methyl-*tert*-butylether (MTBE), where the homopolymer would behave in the exclusion mode and the molar mass decrease is noticed by the shift to higher retention volumes, as illustrated in **Figure 2**.

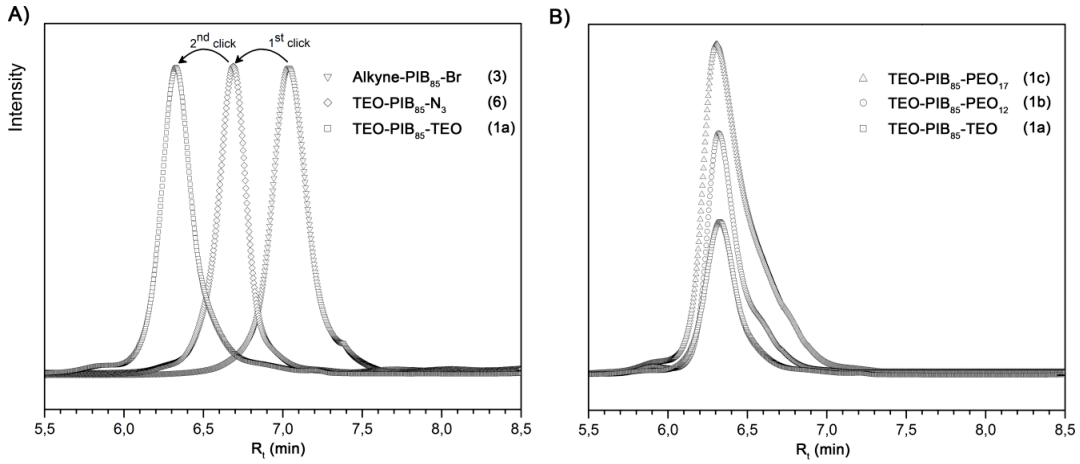


FIGURE 3. LC traces, measured at critical conditions of PIB at a flow rate of 0.3 mL/min, T = 30°C of (A) α-alkyne-ω-bromo-telechelic PIB (3), α-TEO-ω-azido-telechelic PIB (6) obtained after the 1st-"click"-reaction and the final triblock copolymer (1a) after the 2nd "click"-reaction demonstrating a stepwise shift to lower retention times (R_t) with increasing their hydrophilicity by attaching polar PEO-blocks. (B) The final triblock copolymer (1a-c) shows the same main Rt with peak broadening to higher R_t by upon increasing the PEO-block length.

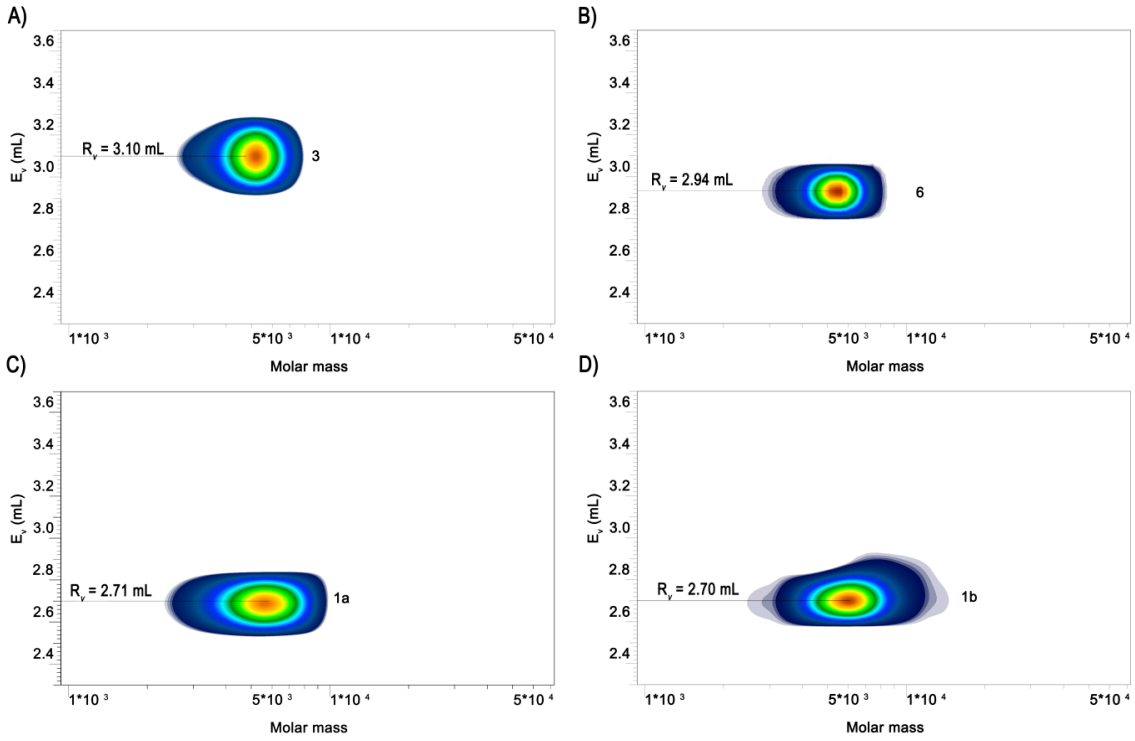


FIGURE 4. 2D-LC/SEC plots of (A) the α-alkyne-ω-bromo-telechelic PIB (3) used as starting material, (B) the TEO-ω-azido-telechelic PIB (6) obtained after 1st "click"-reaction and (C, D) the final triblock copolymer (1a and 1b) after the 2nd "click"-reaction measured under LCCC for PIB in MTBE/MeOH using a weight percent mixture of 85.34/14.66.

In order to enable two-dimensional liquid chromatography as a tool for the characterization of the "click"-reaction steps towards the synthesis of the tri-BCPs, the symmetric and nonsymmetric α-TEO-ω-PEO telechelic PIBs **1a-c** were inspected via LC/SEC and the final products were successfully fingerprinted by MALDI-TOF MS. As a first step towards a successful LCCC-chromatography, the critical conditions of polyisobutylene-homopolymers (PIB) were investigated, using bisallyl-telechelic polyisobutylenes as model-systems (M_n = 3000 (M_w/M_n = 1.2), 9000 (M_w/M_n = 1.1) and 29 300 (M_w/M_n = 1.2) g·mol^{-1}) on a reversed phase column (*Atlantis-RP C18*). Upon the addition of a non-solvent such as methanol to the main elution mobile phase (methyl-*tert*-butylether) up to 10.56% *(w/w)*, the interaction of the PIB-homopolymer with the stationary phase increases, resulting in raised enthalpic interactions, which still is insufficient to eliminate the entropic effect. Nevertheless, by further increasing the amount of the non-solvent to 19.56% *(w/w)*, we observed the adsorption

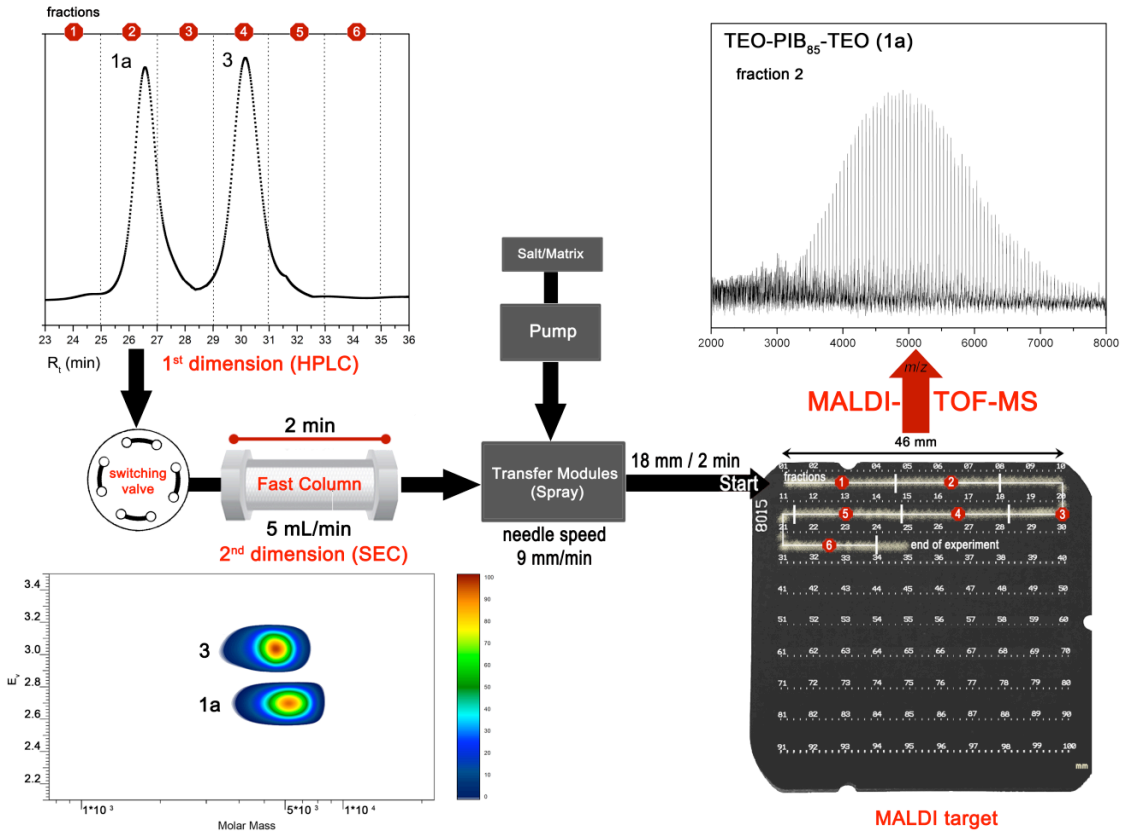


FIGURE 5. Schematic representation of the used chromatographic setup (2D-LC/SEC) coupled to MALDI-TOF mass spectrometry via a transfer modulus sprayer, demonstrated for a mixture of compound 3 and the BCP 1a. Separation of the compounds takes place in the first dimension (HPLC) and after collecting different fractions using a collector/switching valve the second dimension (SEC) is measured. Coupling to MALDI-TOF via transfer modulus allowed a continuous investigation of each fraction at various elution volumes.

chromatography mode (LAC). By fine-tuning the solvent mixtures, the critical conditions of the bisallyl-telechelic-polyisobutylene could be found at the composition of MTBE/MeOH, 84.4/14.6 *(w/w)* (see **Figure 2**). After setting up the critical conditions (LCCC) of PIB using a reverse phase column (*Atlantis-RP C18*), poly(ethylene oxide) would plainly display an entropic behavior and therefore, is expected to exhibit separation in the SEC mode. Hence, increasing the hydrophilic moiety (PEO, from n = 3 to n= 17), would allow the α-TEO-ω-PEO telechelic PIBs (**1a** – **c**) to elute faster. Consequently, the PIB-precursors **3** and **6**, as well as the di- and tri-block copolymers (**1a** – **c**) synthesized via stepwise "click"-reactions could now be investigated under the obtained critical conditions (see **Figure** 3). Accordingly, chromatographic traces of the precursor alkyne-functionalized polyisobutylene (**3**), TEO-PIB_{85}-N_3 di-BCP (**6**) and TEO-PIB_{85}-TEO triblock copolymer (**1a**), were recorded individually via LCCC. As shown in **Figure 3A**, increasing the hydrophilic composition by adding up one polar moiety (TEO) allowed the TEO-PIB_{85}-N_3 diblock copolymer (**6**) to elute faster (R_t = 6.71 min) than the alkyne-functionalized polyisobutylene (**3**) which eluted at R_t = 7.13 min. Correspondently, increasing further the hydrophilicity of the BCPs by attaching an additional poly(ethylene oxide) unit via a second "click"-reaction allowed the tri-BCPs (**1a**) to elute significantly earlier (R_t = 6.33 min) compared to its precursors due to the reduced interaction between the polymer molecules and the hydrophobic stationary phase. Ipso facto, an excellent separation of the PIB-polymers **1a**, **3** and **6** under the given conditions could be achieved by attaching longer chains of PEO (n = 12 or 17) to the ω-end of the tri-BCP **1b** and **1c**, we expected that a shift of the elution volume towards lower retention times should be observed caused by the further increase in the polymer hydrophilicity. However, the increase of the PEO unit up to n = 17, showed no noticeable shift of the tri-BCPs retention times, as listed in **Table 1**. In **Figure 3B**, it is significant to notice that the increase of the full width at half maximum (FWHM) elution peak in the case of the tri-BCPs **1b** and **1c** increases significantly in comparison to the tri-BCP **1a**, due to the additional increase of the PEO chain units (polydispersities). As it turns out, that a direct discrimination between the symmetric and nonsymmetric tri-BCPs can only be achieved, if a supplemental set of information from the mass of the molecules is enabled. Therefore, two-dimensional chromatography was coupled to mass spectrometry to identify the tri-BCPs in question.

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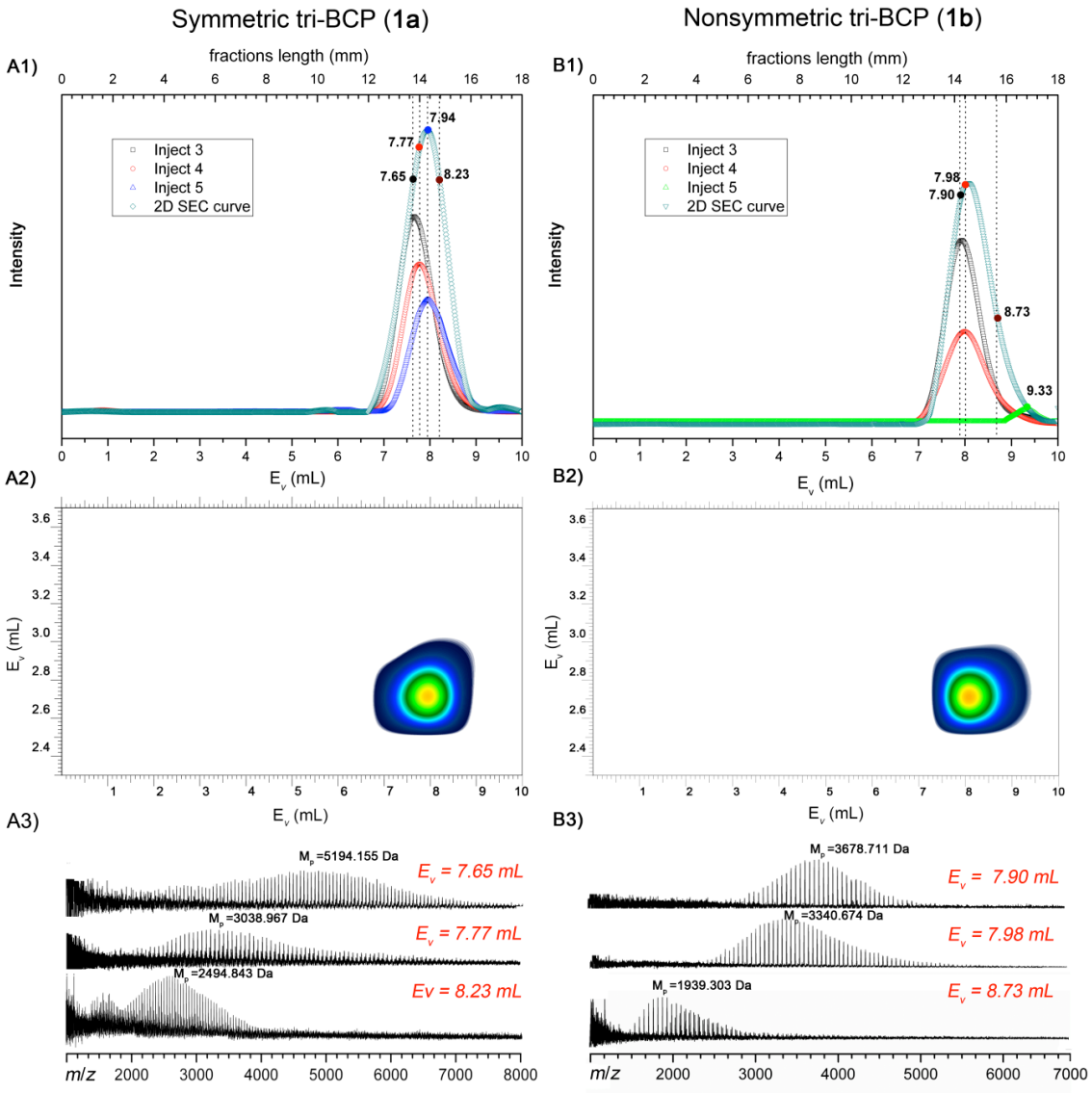

FIGURE 6. 2D-LC/SEC measurements of the symmetric tri-BCP 1a (A1-3) and the nonsymmetric tri-BCP 1b (B1-3) coupled to MALDI-TOF MS allowing the continuous monitoring of the chemical structure at different elution volumes (E_v). (A1 and B1) the recorded 2D-SEC curves with the single SEC curves of each injected fraction, (A2 and B2) the corresponding 2D-LC/SEC plots and in (A3 and B3) the obtained MALDI-TOF spectra at different E_v proving the final chemical structures.

Two-Dimensional liquid chromatography measurements (2D-LC/SEC). In a first step, the successful transformation of α-alkyne-ω-bromo-telechelic PIB (M_n = 4920 g·mol^{-1}) (**3**) to α-TEO-ω-azido-telechelic PIB (**6**) can be monitored by the 2D setup (LC/SEC) using the previously developed critical conditions of the bisallyl-telechelic PIBs. The 2D contour plot (see **Figure 4A-B**) shows no detectable amounts of alkyne-residues (R_t = 31 min, see **Table 1**) in compound (**6**), which elutes at (R_t = 29 min) after extensive purification. Additional 2D measurements were conducted before the purification step (see synthetic part), which allowed to detect that approx. 8*wt*% residual of compound (**3**) (see **Figure S8**, Supplementary) were present in the initially non-purified product. This again demonstrates the high efficiency of the 2D setup (LC/SEC). In the second "click"-reaction, an additional polar moiety (PEO with various chain lengths, n = 3 to 17) was attached on the ω-position of the α-TEO-ω-azido-telechelic PIB (**6**) which increased further the hydrophilicity of the polymer molecules leading again to a shift to earlier retention times of the final tri-BCPs (R_t ~ 27 min, see **Table 1**). In the 2D contour plots of the tri-BCPs (**1a** - **b**, **Figure 4C** and **D**), the results of the 2D separation of tri-BCPs as presented in **Figure 4** are shown, indicating no noticeable shift in the 2D contour diagram when increasing the PEO units in the tri-BCP composition from 4 to 12 units. Even by further increasing the polarity of 12 and 17 units of PEO were insufficient to distinguish the symmetric tri-BCP (**1a**) in the first dimension (LC, see **Figure 3B**) from the nonsymmetric tri-BCPs (**1b** and **c**). Therefore, a direct coupling after the second dimension (SEC) with MALDI-TOF MS to distinguish between the final chemical structures of different BCPs was essential.

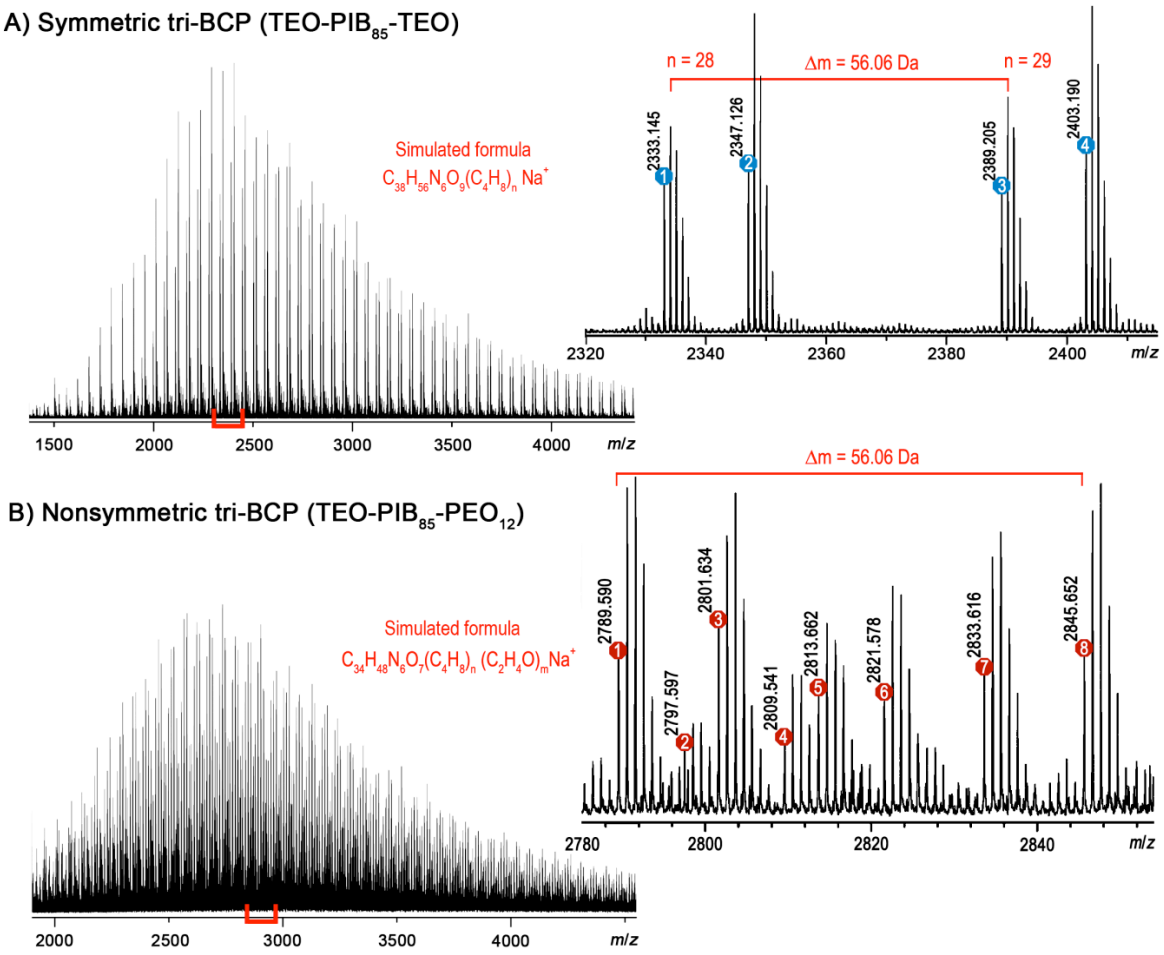

FIGURE 7. MALDI-TOF MS characterization of the symmetric BCP 1a (A) and the nonsymmetric BCP 1b (B) recorded after separation in the second dimension (2D-LC/SEC-MALDI-TOF MS). The obtained MALDI-TOF spectra showing different m/z series with $\Delta m/z$ = 56.06 g·mol^{-1}, which were successfully identified and assigned to the final copolymer structures (see Table 2 and 3), proving the chemical composition and purity of the investigated samples.

Two-dimenional chromatography coupled to mass spectrometry (2D-LC/SEC-MALDI-TOF MS). Although the 2D-LC/SEC setup offered a separation in the case of the polymers displaying different polarities (*e.g.* polymer (**3**) and (**6**)), thus depicting the reaction efficiency and purity together with the molar mass distribution (MMD). Therefore, the complexity of the modification of the symmetric (**1a**) and nonsymmetric triblock copolymers (**1b**, **1c**) demanded more complex analytical methods based on mass spectrometry techniques.

Thus, a setup for the coupling of 2D-LC/SEC towards MALDI-TOF was developed, which should allow a direct separation in two-dimensions (size and polarity) and a detection of the individual mass spectrum of the separated polymers. In addition, an extensive information on the purity of the obtained products is envisioned and thus of significant importance. Therefore, the setup of the two-dimensional chromatography is also used as a tool to depict the efficiency and the purity of all reaction steps. Consequently, in the first step, a mixture of the alkyne-telechelic polyisobutylene (**3**) and α,ω-TEO-telechelic PIB (**1a**) were analyzed as demonstrated in **Figure 5** to investigate the separation and in consequence, prove of the final structure of the block copolymers. Based on the critical conditions of the PIB-homopolymers (3 to 30 kg·mol^{-1}), the LC trace of the mixture (**1a** and **3**), as shown in **Figure 5** represent the number of fractions calculated for one dimension. Subsequently, the loop volume (0.2 mL x 2 loops) connected to the switching valve allows each fraction to continuously transfer into a highspeed SEC column running at a flow rate of 5 mL/min. Therefore, molecules are separated based on their hydrodynamic volume and their average molar mass M_n can be calculated. Furthermore, each fraction eluting from the highspeed column has a retention time of two minutes (E_v = 10 mL), which can be consequently conveyed to a sample transfer module (TM sprayer). The upcoming stream is continuously mixed with a mixture of LiTFA/DCTB (20 mg/mL) and further sprayed on a MALDI-target, where each fraction has a defined track length (46 mm). Therefore, taking advantage of an automated experiment, one can predetermine the number of required tracks that would cover the number of fractions of the second dimension. Hence, we were able to directly correlate the recorded MS spectra with the previous SEC fractions.

Table 2. Experimental results of 2D-LC/SEC offline coupled to MALDI-TOF MS of symmetric tri-BCP (**1a**)

entry	polymer structure[a]	species	*m/z* found [g·mol^{-1}]	*m/z* simulated [g·mol^{-1}]	error[ppm]
1	n = 28	$[C_{150}H_{280}N_6O_9]$ Na^+	2333.145	2333.153	6.1
2	n = 29(2)[b]	$[C_{151}H_{282}N_6O_8]$ K^+	2347.126	2347.148	9.3
3	n = 29	$[C_{154}H_{288}N_6O_9]$ Na^+	2389.205	2389.216	6.0
4	n = 30(2)[b]	$[C_{155}H_{290}N_6O_8]$ K^+	2403.190	2403.210	8.4

[a]$\Sigma = C_{38}H_{56}N_6O_9(C_4H_8)n$. [b]Fragmentation ($-C_2H_4OCH_3$).

Table 3. Experimental results of 2D-LC/SEC offline coupled to MALDI-TOF MS of nonsymmetric tri-BCP (**1b**).

entry	polymer structure[a]	species	*m/z* found [g·mol^{-1}]	*m/z* simulated [g·mol^{-1}]	error[ppm]
1	n = 33; m = 6	$[C_{178}H_{336}N_6O_{13}]$ Na^+	2789.590	2789.571	7.0
2	n = 30; m = 10	$[C_{174}H_{328}N_6O_{17}]$ Na^+	2797.597	2797.488	6.5
3	n = 34; m = 5	$[C_{180}H_{340}N_6O_{12}]$ Na^+	2801.634	2801.607	9.7
4	n = 31; m = 9	$[C_{176}H_{332}N_6O_{16}]$ Na^+	2809.541	2809.524	5.9
5	n = 35; m = 4	$[C_{182}H_{344}N_6O_{11}]$ Na^+	2813.662	2813.644	6.6
6	n = 32; m = 8	$[C_{178}H_{336}N_6O_{15}]$ Na^+	2821.578	2821.561	6.3
7	n = 33; m = 7	$[C_{180}H_{340}N_6O_{14}]$ Na^+	2833.616	2833.597	6.6
8	n = 34; m = 6	$[C_{182}H_{344}N_6O_{13}]$ Na^+	2845.652	2845.633	6.5

[a]$\Sigma = C_{34}H_{48}N_6O_7(C_4H_8)_n(C_2H_4O)_m$

After drying, the MALDI-target was inserted into a MALDI-TOF MS to identify the chemical composition and the final structure of the respective polymers. Matrix-assisted laser desorption ionization - time of flight mass (MALDI-TOF MS) offers a high selectivity and allows desorption of a large variety of molecules. The evaluation of the molecular mass provided by MALDI-TOF-MS. However, does not serve to usefully analyze synthetic polymers with a broader molecular weight-distribution. Therefore, a fractionation of highly polydisperse polymers via size exclusion chromatography (SEC), thus providing a narrow molar mass distribution (MMW) for each fraction, opens the possibility to combine the information collected by two-dimensional chromatography and mass spectrometry (MALDI-TOF MS) in a useful manner. As shown in **Figure 6**, fractions collected from the second dimension (SEC) are further analyzed in depth by MALDI-TOF MS as a mass detection method leading to a continues monitoring of the chemical structure at each elution volume.

Figure 6A1 and **B1** represent single SEC curves recorded from different injections (collected fractions) using an evaporative light scattering detector (ELSD) directly coupled to the SEC. The 2D curve renders the overall graphical representation of the molar mass extracted from the intensities of all injections achieved throughout the 2D-measurements. The 2D contour plot (see **Figure 6A2** and **B2**) of the symmetric (**1a**) and nonsymmetric (**1b**) tri-BCPs is then directly correlated to the 2D SEC curves. Each injection and its corresponding fraction is continuously sprayed onto a MALDI-target via a sprayer transfer module (TM sprayer). The run time of one single SEC measurement takes 2 min (E_v = 10 mL) and thus represents a fraction sized 18 mm on the MALDI-target. To correlate the monitored masses at each specific elution volume with a chemical structure that proves the desired molecular architecture of the investigated samples, MALDI-TOF MS were performed as depicted in **Figure 6A3** for the symmetric tri-BCP (**1a**) and in **6B3** for the nonsymmetric polymer (**1b**). The monitored masses by MALDI-TOF MS with increasing elution volume confirmed the SEC behavior of the analyzed BCPs.

The recorded MALDI-TOF spectra in **Figure** 7 unveiled the functionality type distribution (FTD) of the tri-BCPs in question. The symmetry of the tri-BCP α,ω-TEO-telechelic PIB (**1a**) in **Figure** 7A is depicted by the assignment of the main species $[C_{38}H_{56}N_6O_9(C_4H_8)_{28}]Na^+$, the monoisotopic peak found *m/z* = 2333.145 g·mol^{-1} agrees with the simulated structure *m/z* = 2333.153 g·mol^{-1} with an error of 6.1 ppm, see **Table** 2. The species $[C_{151}H_{282}N_6O_8]$ K^+ (*m/z* = 2347.126 g·mol^{-1}), as observed in MALDI-TOF MS spectra could be assigned to the main peak fragmentation of the last $-(C_2H_4OCH_3)$ unit. Even though a potassium salt was not intentionally added as cationization agent, the potassium ion associated with homologues distribution could still be observed as contamination in glasswares and solvents due to the fact that potassium hydroxide was applied to predry solvents which were used in the reported synthetic pathway, similar to reports by other authors investigating PEO-polymers via MALDI-TOF MS.[87, 88] Furthermore, the delayed ion extraction technique enables more time for cation attachment based on the affinities of polymer samples.[87]

The nonsymmetric tri-BCP α-TEO-ω-PEO-telechelic PIB (**1b**) was also analyzed in 2D-LC/SEC-MALDI-TOF MS. The additional PEO unit, that exhibits a polydispersity compared to TEO chain end leads to a much more complex MALDI spectrum, showing several side series

characterized by the variation of PEO units (4 to 10), as shown in **Table 3**.

The chain length distribution (CLD) extracted from **Figure** 7B, show a main series with 6 PEO units. Hence, the main monoisotopic peak, m/z = 2789.590 g·mol^{-1} corresponds to the structure $[C_{34}H_{48}N_6O_7(C_4H_8)_{33}(C_2H_4O)_6]Na^+$ which agrees with the simulated isotopic pattern, (compare **Table 3**). Nevertheless, two different patterns would describe the PEO distribution in the triblock copolymer α-TEO-ω-PEO-telechelic PIB (**1b**), the decrease of PEO unit (n = 6, 5 and 4) with increasing PIB chain, **Figure 7B** (label 1, 3 and 5) and the other pattern which can be distinguished by increasing the content of the PEO (n = 6, 7, 8, 9 and 10) with decreasing in the PIB content, **Figure 7B** (label 8, 7, 6, 4 and 2). The latter pattern shows a broader chain length distribution (CLD) characterized by a significantly reduced ionization efficiency (intensity) compared to the first pattern, in which stronger and narrow distribution can be observed. **Table 3**, clearly demonstrate well-resolved isotopic patterns of the nonsymmetric triblock copolymer alongside of PEO chain length distribution (CLD).

CONCLUSION

In this publication, a direct coupling between two-dimensional LC/SEC under LCCC conditions with MALDI-methods was investigated for the analysis of symmetric (**1a**) and nonsymmetric (**1b**, **1c**) PIB-PEO based triblock copolymers. A series of two stepwise azide/alkyne-"click"-reaction after living carbocationic polymerization based on methylstyrene epoxide as initiator was used to prepare the either symmetric (**1a**) or nonsymmetric (**1b**, **1c**) PIB-PEO based triblock copolymers (PEO_m-PIB-PEO_n BCPs (with m=3; n=3, 12 or 17)). Demonstration of the efficiency of the "click"-reaction and the purity of the final triblock copolymers was accomplished by searching for the critical conditions of the PIB-homopolymers (M_n = 3 to 30 kg·mol^{-1}) in the isocratic elution mode (LCCC). Using a reverse phase *Atlantis-RP C18* column, it was possible to separate the final polymers from intermediate polymers using a mixture of methyl-*tert*-butyl ether/methanol (85.34/14.66 (*w*/*w*)) as the mobile phase. Based on the PEO segment length and the overall hydrophobicity of the tri-BCPs, we observed a complete separation of the stepwise "click"-products. Final analysis of the different block copolymers together with the molecular weight was then accomplished by direct coupling of the 2D-LC/SEC to (MALDI-) mass spectrometry via a spray-module, which was directly connected after the 2D-LC/SEC-system. Thus, enabling to resolve the so achieved separation into a series of time-correlated MALDI-spectra. A clear identification of the symmetric (**1a**) and nonsymmetric (**1b**, **1c**) triblock copolymers could be accomplished, additionally allowing to identify all pre-reaction steps thus proving the structure and purity of the final symmetric and nonsymmetric triblock copolymers. The presented methodology allows to achieve a true picture of the purity of block copolymers after linkage or direct crossover reactions – an aspect which is important for use of these polymers as drug-delivery vesicles, most of all in the investigation of polymersomal membranes, formed from such or similar block copolymers.

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ABBREVIATIONS

BCP, block copolymer; **CLD**, chain length distribution; **DAD**, diode array detector; **ELSD**, evaporative light scattering detector; **FTD**, functionality type distribution; **LAC**, liquid adsorption chromatography; **LC**, liquid chromatography; **LCCC**, liquid chromatography at critical conditions; **LCCP**, living carbocationic polymerization; **MALDI-TOF MS**, matrix assisted laser desorption ionization – time of flight – mass spectrometry; **MeOH**, methanol; **MTBE**, methyl-*tert*-butyl ether; **MWD**, molar weight distribution; $\mathbf{M_n}$, number average molar mass; **PEO**, poly(ethylene oxide); **PIB**, polyisobutylene; **RI**, refractometric detector; **RP-C18**, Atlantis reversed phase column; **SEC**, size exclusion chromatography; **TEO**, triethylene oxide; **TM sprayer**, transfer module sprayer; **THF**, tetrahydrofuran; **2D**, two-dimensional chromatography; **2D LC/SEC-ESI-TOF-MS**, liquid chromatography coupled in parallel to size exclusion chromatography and ESI-TOF-MS; **2D LC/SEC**, LC coupled to SEC; **2D LC/SEC-(MALDI)-TOF MS**, LC coupled in series to SEC and MALDI-TOF MS (via TM-sprayer).

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SUPPORTING INFORMATION

SYNTHESIS

1. Synthesis of a-hydroxymethyl-w-bromo telechelic PIB (**2**).

A representative procedure for the preparation of a-hydroxymethyl-w-bromo telechelic PIB (**2**) with M_n = 5,000 g.mol^{-1} was achieved by a combined method of Puskas[1] and Storey[2] as published previously by our group.[3] Under an argon atmosphere, dichloromethane and n-hexane (40/60 mixture), di-*tert*-butyl pyridine (D*t*BP) (0.005 mol/L), and MSE initiator (0.049 mol/L) were cooled down to -60°C in a three-necked round-bottom flask equipped with a septum and a mechanical stirrer. To the mixture was added a solution of $TiCl_4$ (0.034 mol/L) and subsequently the polymerization was started by adding condensed isobutylene (1 mol/L) into the reaction mixture via syringe. After complete conversion of the monomer (~20 minutes), the polymerization mixture was cooled further to -70°C and a quantitative end-capping reaction was achieved by using an excess of 3-bromopropoxybenzene (BPB) (2.5 equiv per chain end). Finally, after 3 hours, the catalyst was destroyed by addition of methanol (large excess), and the polymer was isolated by repeated precipitation from hexane into methanol. The resulting a-hydroxymethyl-w-bromo telechelic PIB (**2**) was obtained in a yield of 98%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 6.82 (d, J = 8.79 Hz, 2H), 4.08 (t, J = 5.78 Hz, 2H), 3.64 (d, J = 10.77 Hz, 1H), 3.58 (t, J = 6.53 Hz, 2H), 3.41 (d, J = 10.78 Hz, 1H), 2.29 (t, J = 6.23 Hz, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) 154, 148.5, 141.6, 128.4, 125.7, 125.1, 114, 70.1, 67.3, 53.4, 43.8, 32.6, 30, 28, 20.5. (^{1}H- and ^{13}C- NMR data represent the end group structure of the polymer chains).

Table S1. Experimental results of isobutylene polymerization using MSE/$TiCl_4$ as initiator system.

Entry	Sample	M_n[1)] (theory)	Characterization				Yield [%]
			M_n[1)] (GPC)	M_w[1)] (GPC)	PDI	M_n[1)] (NMR)	
1	HO-PIB_{57}-Br (**2a**)	3000	3190	4950	1.25	3540	92
2	HO-PIB_{70}-Br (**2b**)	4000	3920	5060	1.29	4260	89
3	HO-PIB_{85}-Br (**2c**)	5000	4760	5980	1.25	5080	94

1) Number average molecular weight in [g/mol].

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FIGURES AND SCHEMES

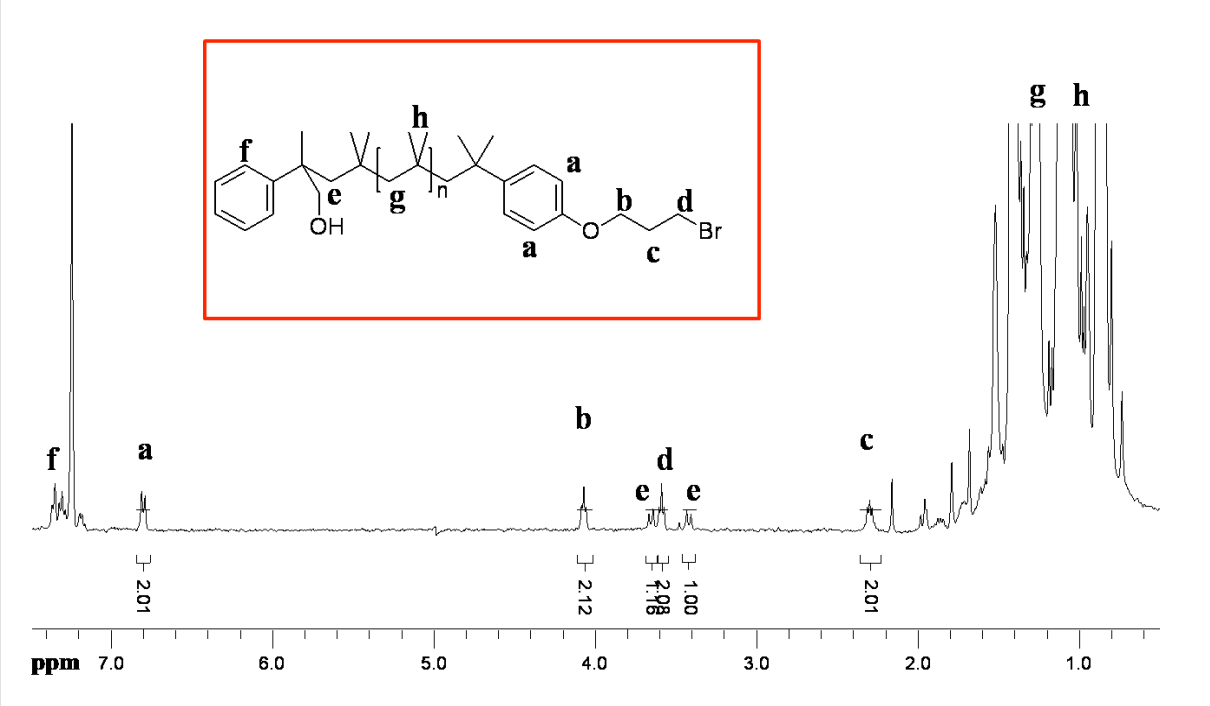

Figure S1. ^{1}H-NMR of α-hydroxyl-ω-bromo-telechelic polyisobutylene (**2**).

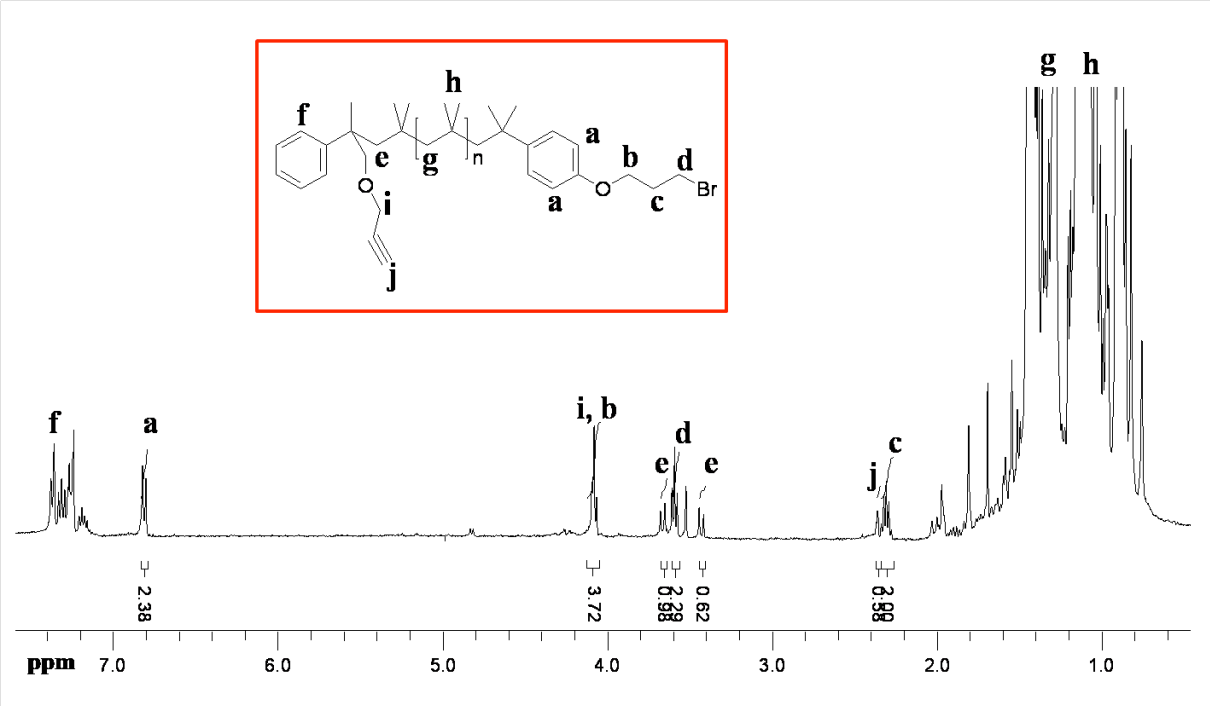

Figure S2. ^{1}H-NMR of α-alkyne-ω-bromo-telechelic polyisobutylene (**3**).

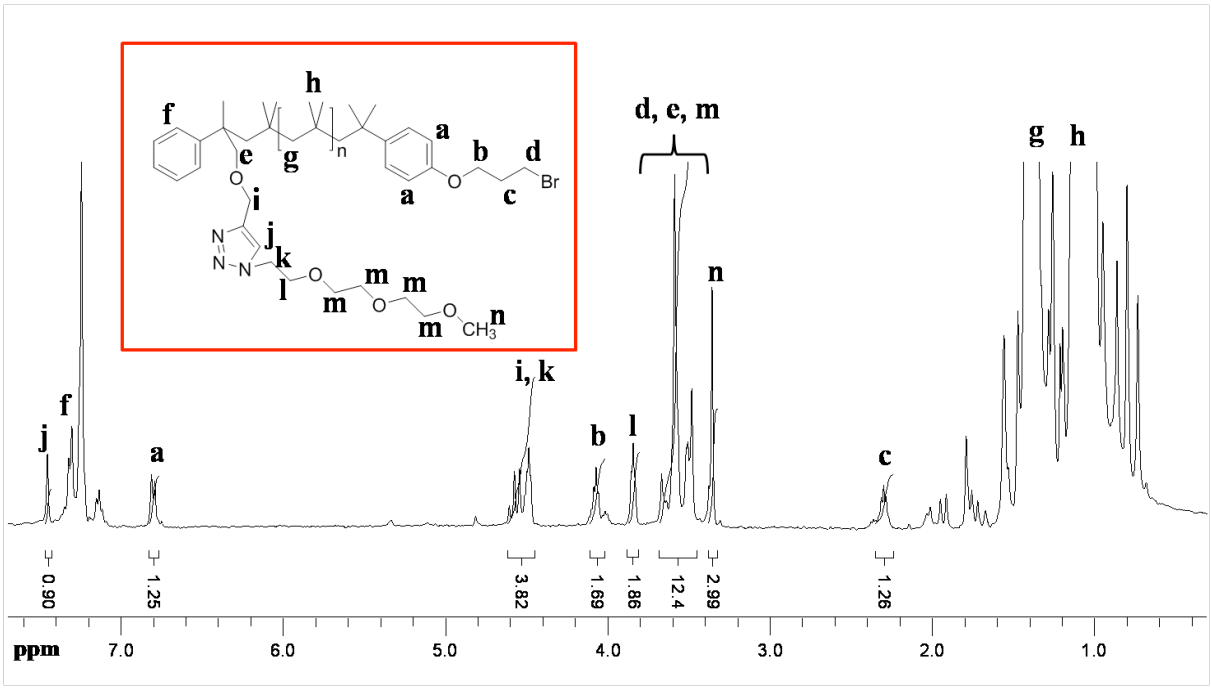

Figure S3. ^{1}H-NMR of α-TEO-ω-bromo-telechelic polyisobutylene (**5**).

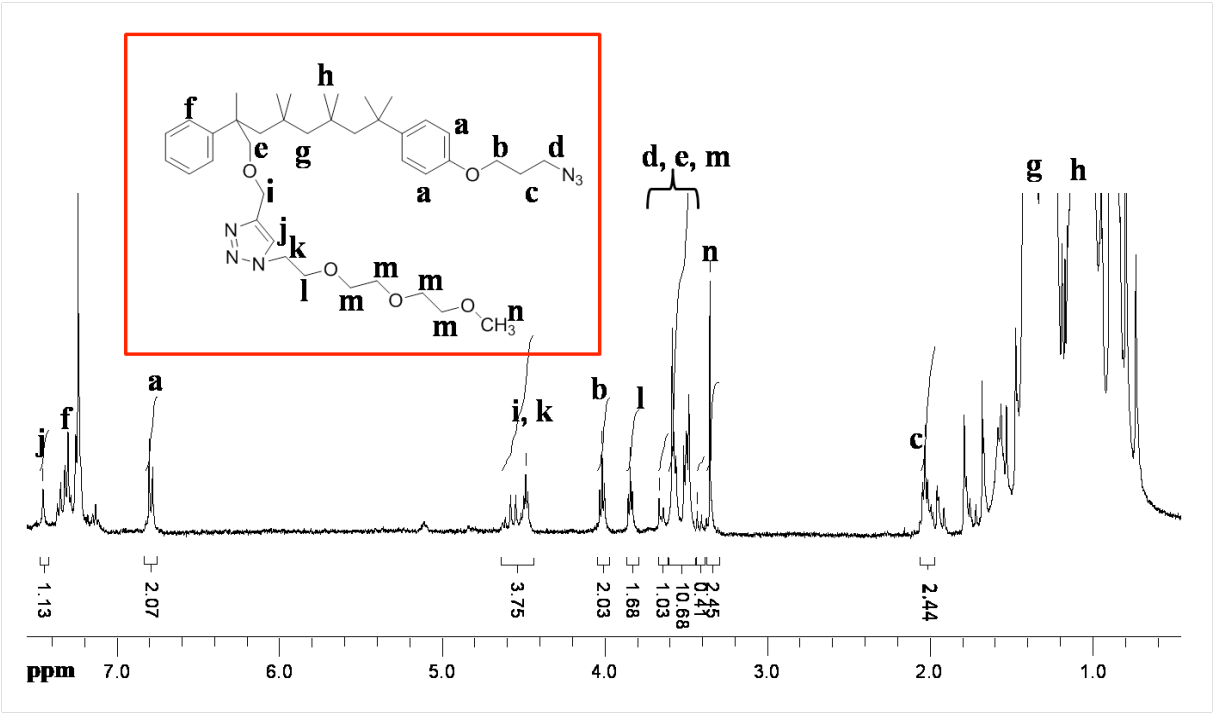

Figure S4. ^{1}H-NMR of α-TEO-ω-azido-telechelic polyisobutylene (**6**).

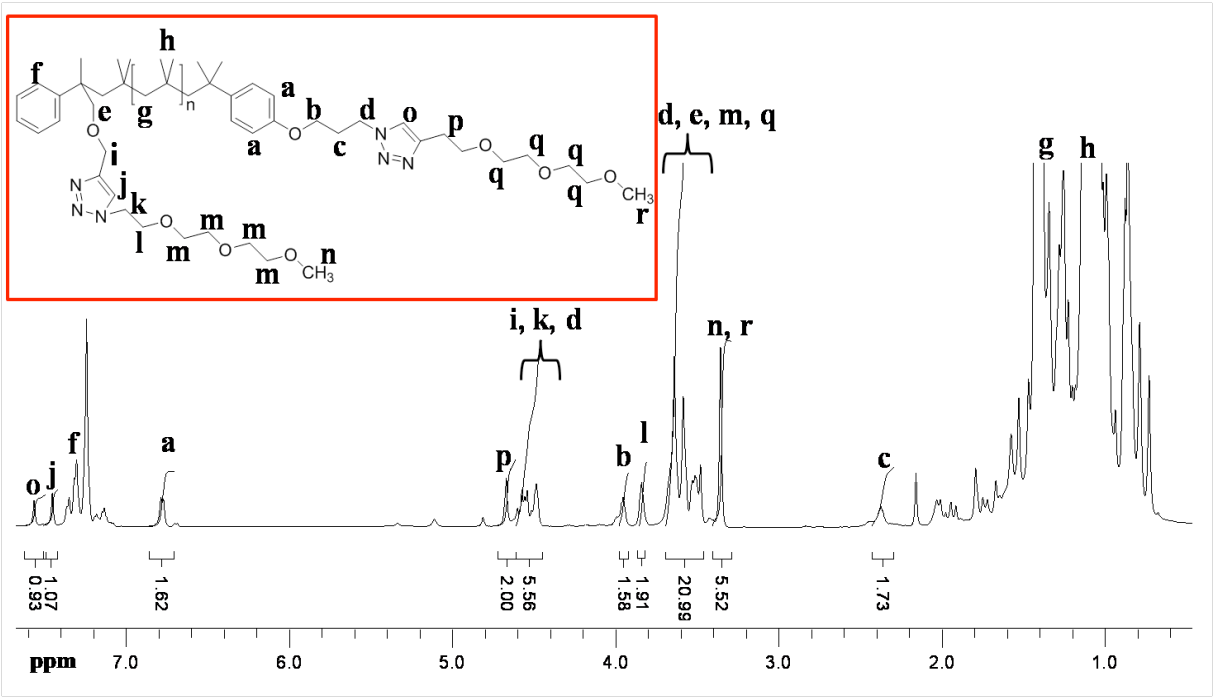

Figure S5. ^{1}H-NMR of symmetric TEO-PIB$_{85}$-TEO block copolymer (**1a**).

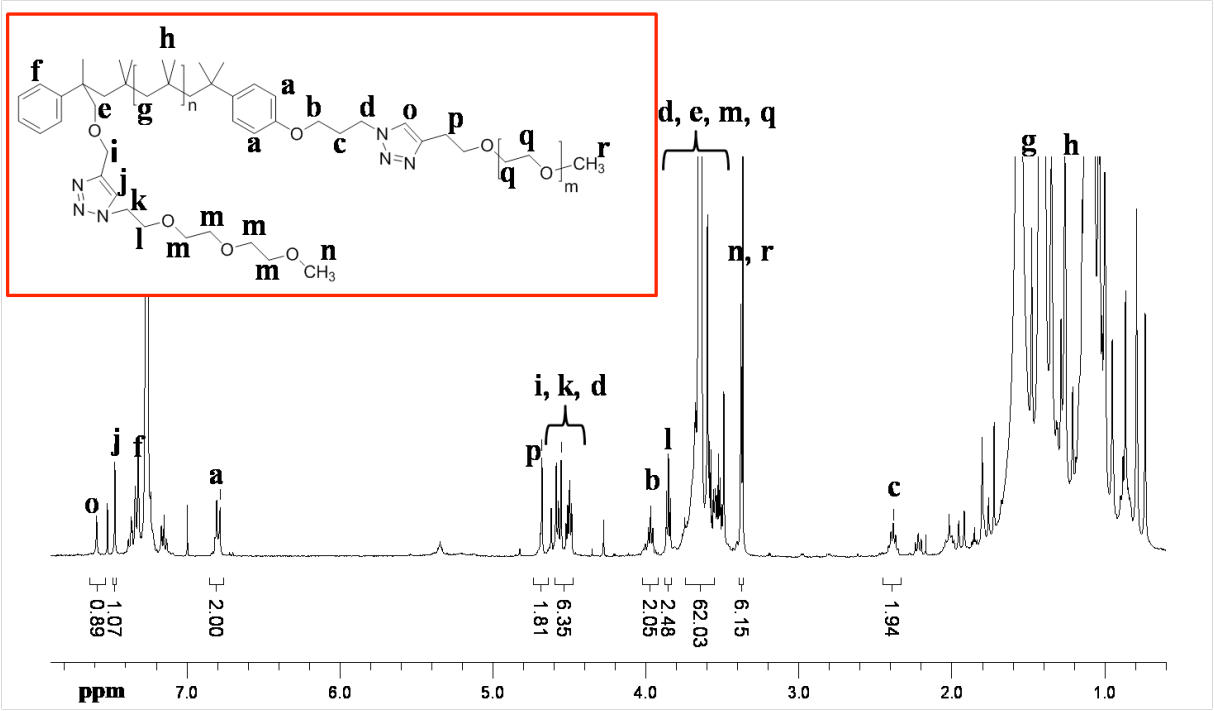

Figure S6. ^{1}H-NMR of nonsymmetric TEO-PIB$_{85}$-PEO$_{12}$ block copolymer (**1b**).

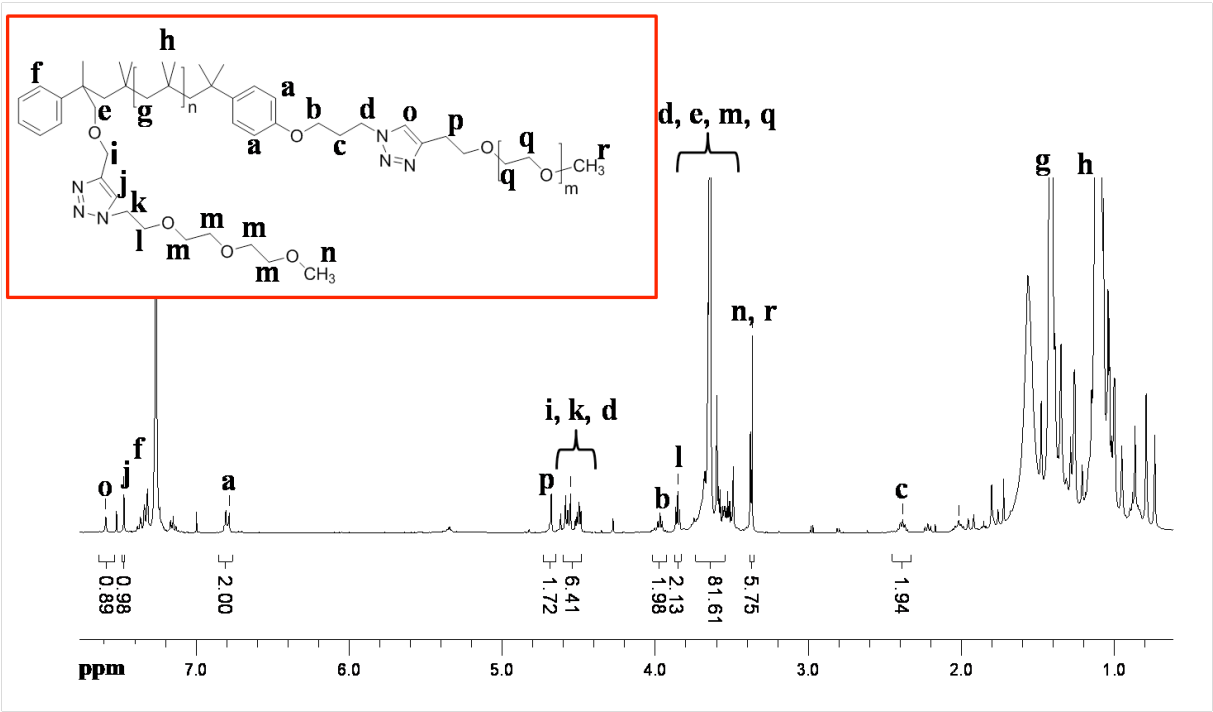

Figure S7. ^{1}H-NMR of nonsymmetric TEO-PIB$_{85}$-PEO$_{17}$ block copolymer (**1c**).

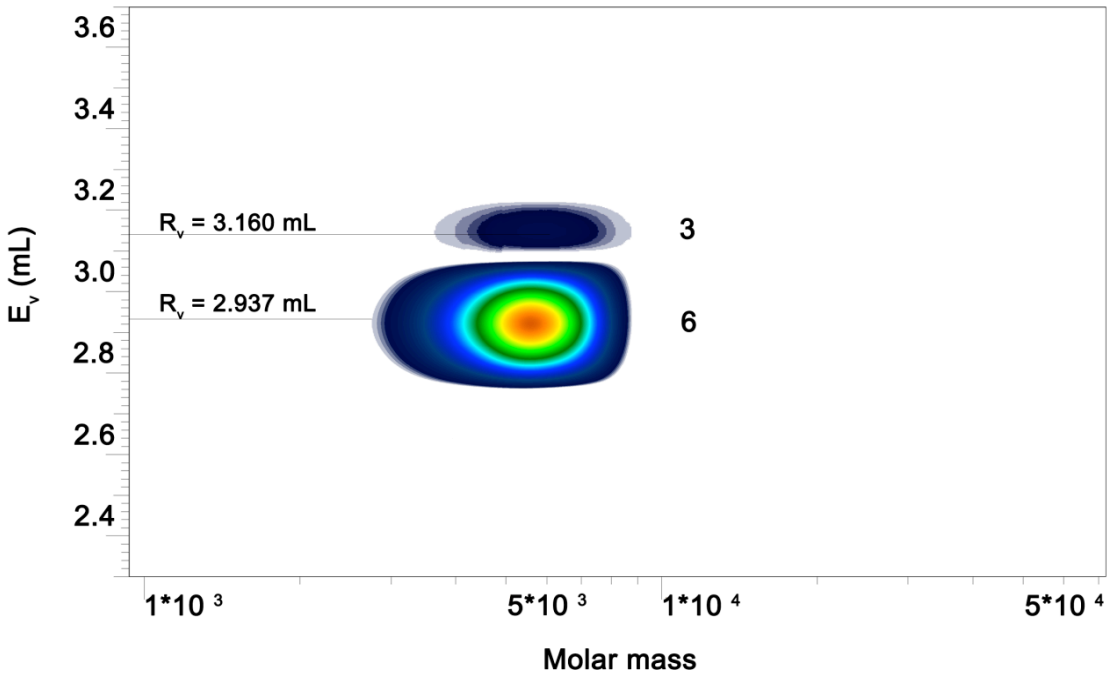

Figure S8. 2D-LC/SEC spectrum of unpurified compound **6** after click-reaction demonstrating same impurities of alkyne-telechelic PIB (**3**).

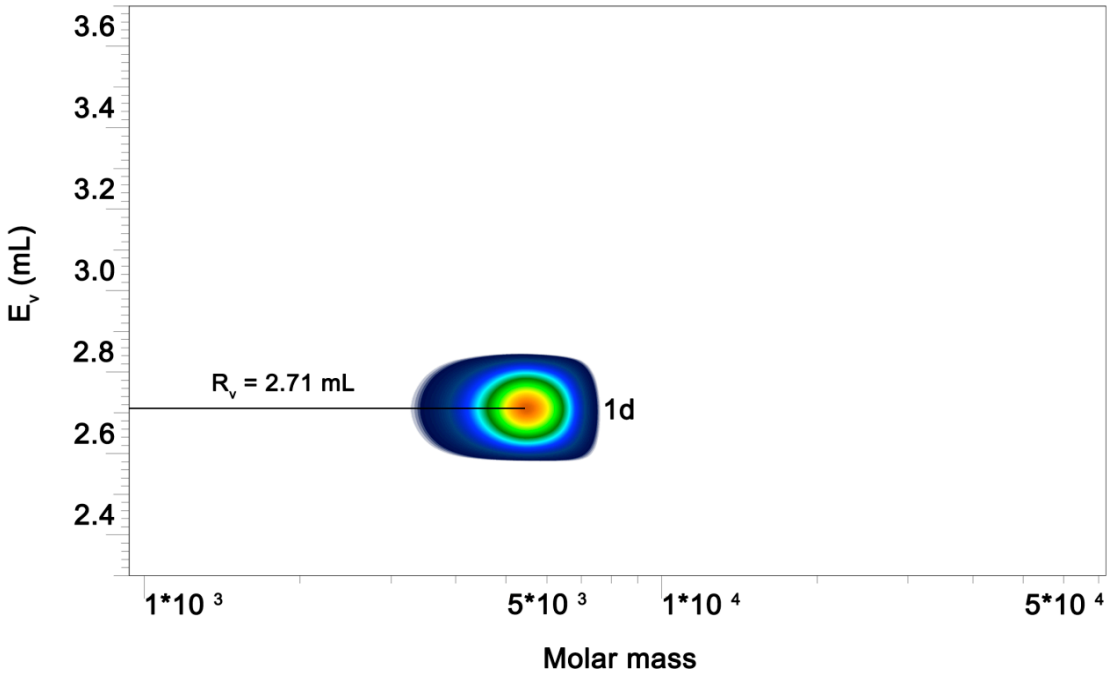

Figure S9. 2D-LC/SEC spectrum of symmetric TEO-PIB_{57}-TEO block copolymer (**1d**).

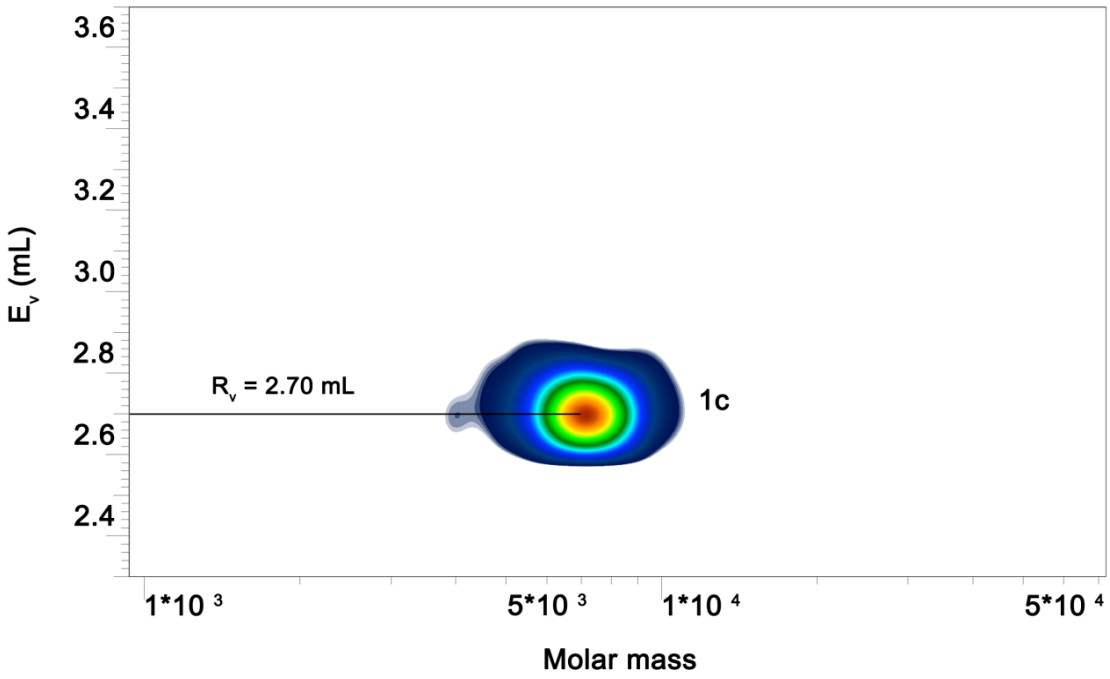

Figure S10. 2D-LC/SEC spectrum of nonsymmetric TEO-PIB$_{85}$-PEO$_{17}$ block copolymer (**1c**).

SUMMARY 4

Functional oligomers, polymers and copolymers represent important material classes, which have the potential to play a key role in various new applications. The properties of these materials pose challenges for structural analysis as traditional characterization techniques fail to put up a better understanding of their structural organization and architecture. Therefore, the primary goal of this dissertation has been focused on the development of new analytical technologies and methodologies based on one- and two-dimensional coupling of chromatographic devices and soft ionization mass spectrometric techniques, as shown in **Figure 6**.

Hence, we were able to report for the first time on the characterization of functional amino resin oligomers, end group modified polymers or block copolymers based on poly(ε-caprolactone)s (pCLs), polyisobutylenes (PIBs) and poly(ethylene oxide)s (PEOs) via a novel homemade setup coupling one-dimensional HPLC online to ESI-TOF MS or two-dimensional LC/GPC online/offline to ESI or MALDI-TOF MS, respectively. Herein, different chromatographic conditions (*e.g.* varying the stationary phase, mobile phase, column temperature or elution mode) were systematically altered in order to achieve a sufficient separation of these complex samples.

Online coupling of one-dimensional LC to ESI-TOF mass spectrometry unveiled a full and direct analysis of oligomeric melamine-formaldehyde resin species (see **chapter 3.1** of *results and discussion* based on the publication: *J. Polym. Sci., Part A*, **2010**, 48, 4855-4866). Whereas, a two-dimensional coupling between HPLC (DAD detector) in either reversed or normal phase mode to GPC (ELSD detector), provided detailed information about the chemical functionality combined with the molecular size of the investigated complex polymer samples (see **chapter 3.2** and **3.3** of *results and discussion* based on the publications: *Macromolecules*, **2012**, 45, 9779-9790 and *Macromolecules*, **2013**, 46, 7638-7649, respectively). The fractions conveyed from the second dimension, were split to ESI-TOF MS (direct coupling) and MALDI-TOF MS (transfer module sprayer), as illustrated in **Figure 6**.

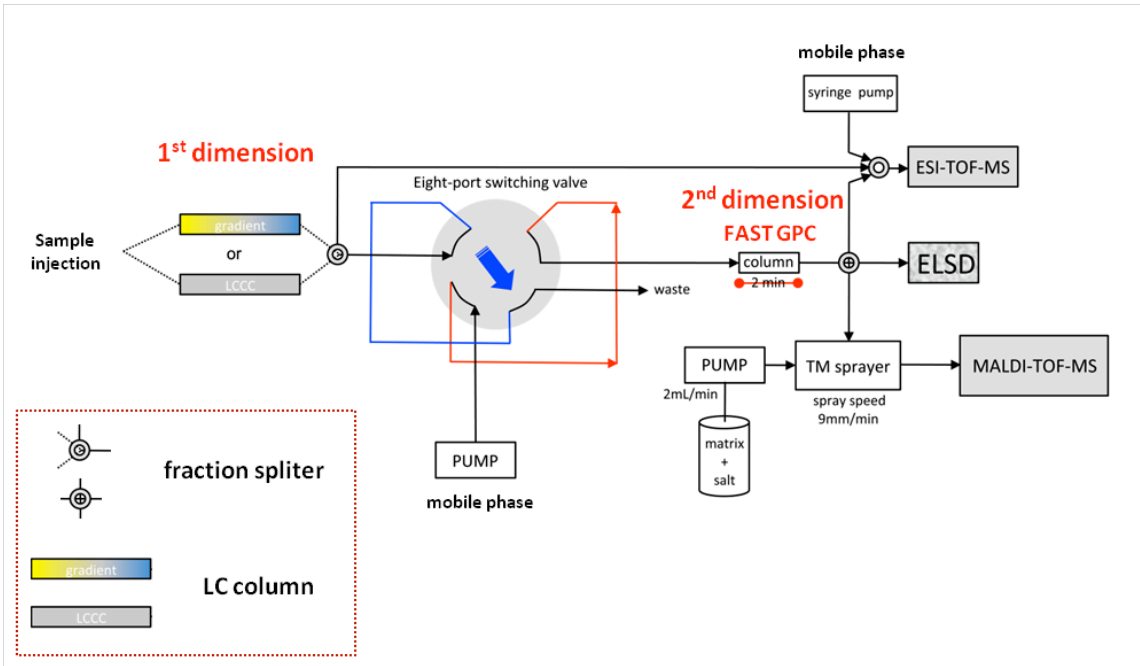

Figure 6. Schematic representation of the developed multidimensional liquid chromatographic setup coupled to soft-ionization techniques, offline to MALDI-TOF MS via transfer module sprayer and online to ESI-TOF-MS.

For one-dimensional characterization methods utilizing liquid chromatography and soft ionization techniques (ESI-TOF MS), amino resins oligomers were separated on a reversed phase columns and their chemical structures were identified via DAD and ESI-TOF-MS detectors, which provided a simple and robust interface that can be applied for a wide range of substance analysis. Especially, amino resins with functional moieties prepared by using the well-known azide/alkyne-"click"-reaction, could be successfully characterized confirming their exact degree of functionalization.

As demonstrated in **chapter 3.1** of *results and discussion*, the alkyne end group functionality was introduced to melamine-formaldehyde resins by adding propargylic alcohol to MF oligomers allowing a direct cocondensation reaction (see synthetic scheme in **Figure 7**). Structures of the alkyne-modified mono-, bi-, and trinuclear melamine-species were successfully identified via 1D-LC-ESI-TOF MS methods proving the exact amount (mol%) of the covalent functionalization of the MF oligomers with alkyne-moieties.

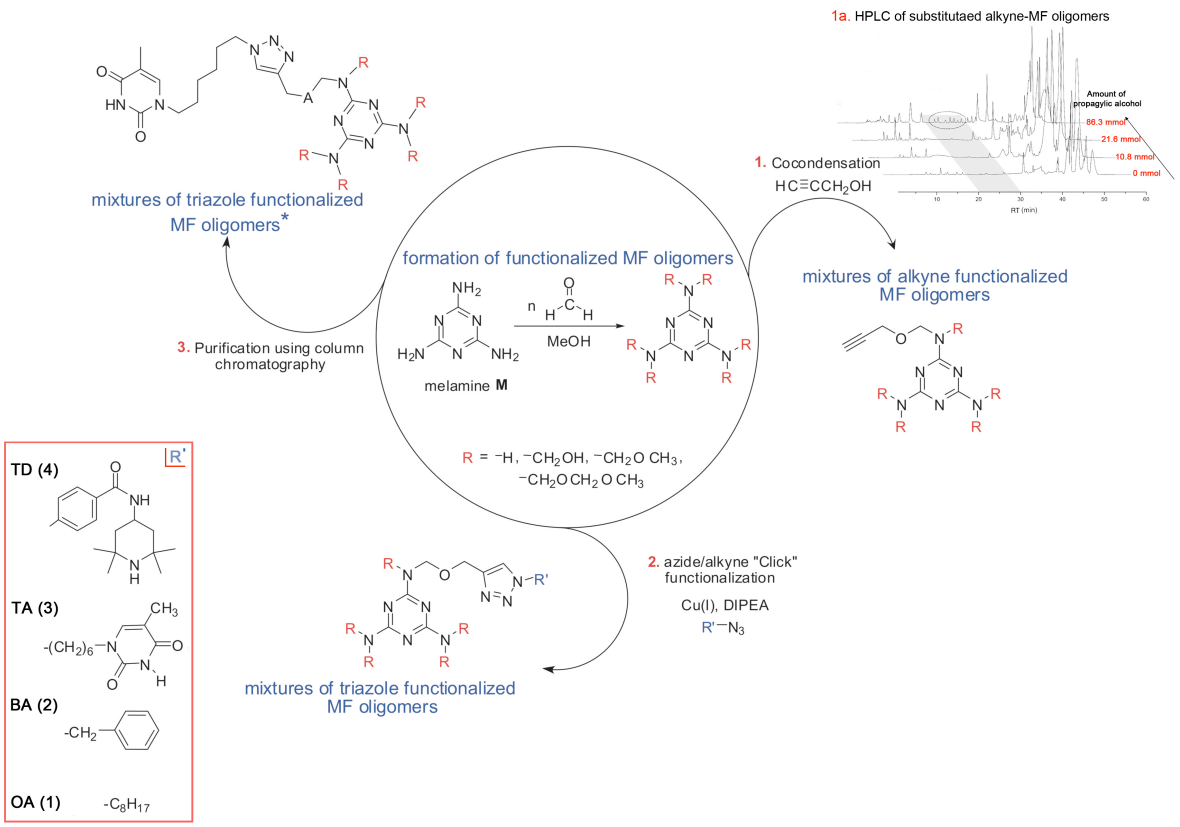

Figure 7. Analysis and characterization of stepwise-modified amino resins via azide/alkyne-"click"-chemistry using the online HPLC-ESI-TOF-MS coupling technique (see publication: Barqawi et al, *J. Polym. Sci., Part A*, **2010**, 48, 4855-4866).

Subsequent modification of the alkyne-modified resin oligomers was accomplished by reaction with functional azides (*e.g.* octyl azide (**1**), (azidomethyl)benzene (**2**), 1-(6-azidohexyl) thymine (**3**), and 4-azido-N-(2,2,6,6-tetramethylpiperidin-4-yl)benzamide (**4**)) using Cu(I)Br and DIPEA to promote the azide/alkyne-"click"-reaction. The formation of triazolyl-modified MF-resins was subsequently confirmed by the 1D-LC-ESI-TOF MS method, indicating a covalent modification of the amino resins with all azido-species (**1** to **4**), as successfully published in *J. Polym. Sci., Part A*, **2010**, 48, 4855-4866.

In contrast to oligomeric mixtures, synthesized multi-step polymers (*e.g.* di- and tri-BCPs) having complex molecular heterogeneities, which make it tedious to clearly identify their exact chemical composition, purity and structural arrangement by one-dimensional (1D-) chromatographic separation. To overcome the limitations of a 1D-separation method in terms of complex molecular heterogeneities in such polymer samples, multidimensional chromatographic methods (*i.e.* LC/GPC) coupled online/offline to mass spectrometric techniques was developed, demonstrating an efficient analytical method for a wide range of functional polymers to clearly identify their final structures and chemical composition.

Thus, end group functionalized poly(ε-caprolactone) polymers prepared via azide/alkyne "click" chemistry were used in order to develop a new LC/GPC coupling technique with ESI- or MALDI-TOF MS proving *e.g.* the efficiency of the "click" reaction with increasing molecular weight. By identifying the impurities within the recorded 2D-chromatograms of all investigated polymer samples, a quantitative analysis proves that the "click"-reaction efficiencies decreases for high-molecular weight pCLs (above 60 kg·mol^{-1}), which was clearly confirmed by identified alkyne-modified pCL residues (see publication: *Macromolecules*, **2012**, 45, 9779-9790). In particular, we have investigated the separation of low molecular weight pCLs ($M_n \leq 35$ kg·mol^{-1}) using a gradient elution chromatographic method (eluent: ACN/THF) monitoring the end group functionalization of the separated polymers online via ESI-TOF and/or offline via MALDI-TOF mass spectrometry, the latter coupled via a transfer module sprayer (TM sprayer). Due to the poor solubility of high molecular weight pCLs (M_n above 35 kg·mol^{-1}), a second chromatographic method was employed, enabling the separation of end group-functionalized pCLs up to a molecular weight of 115 kg·mol^{-1}. To develop a molecular weight independent separation of pCl polymers having the advantage of a simple separation based on the end group functionality (*i.e.* polarity separation), the critical solution conditions of pCls were discovered by varying the eluent composition (mobile phase), chromatographic column (stationary phase) and column temperature. Finally, the critical conditions were optimized on a silica gel modified amino-propyl column (NUC-NH_2) using an isocratic eluent mixture of DCM/THF in a volume ratio of 92/8. Besides the chemical composition revealed by the developed LCCC methods, the coupling to GPC as the second dimension disclosed the molecular weight distribution of all modified pCLs up to M_n of 115 400 g·mol^{-1}.

High-performance liquid chromatography under critical conditions (LCCC) coupled to size exclusion chromatography (2D-LC/SEC) firstly manifested detailed information on the chemical composition and end group structure, and further on the molecular weight distribution of the studied polymeric samples. Considering the 2D-LC/GPC-MS results of pCL homopolymers, this system was used to develop further an efficient separation method examining the specific block composition, purity and structural symmetry of PIB-PEO based block copolymers (see publication: *Macromolecules*, **2013**, 46, 7638-7649).

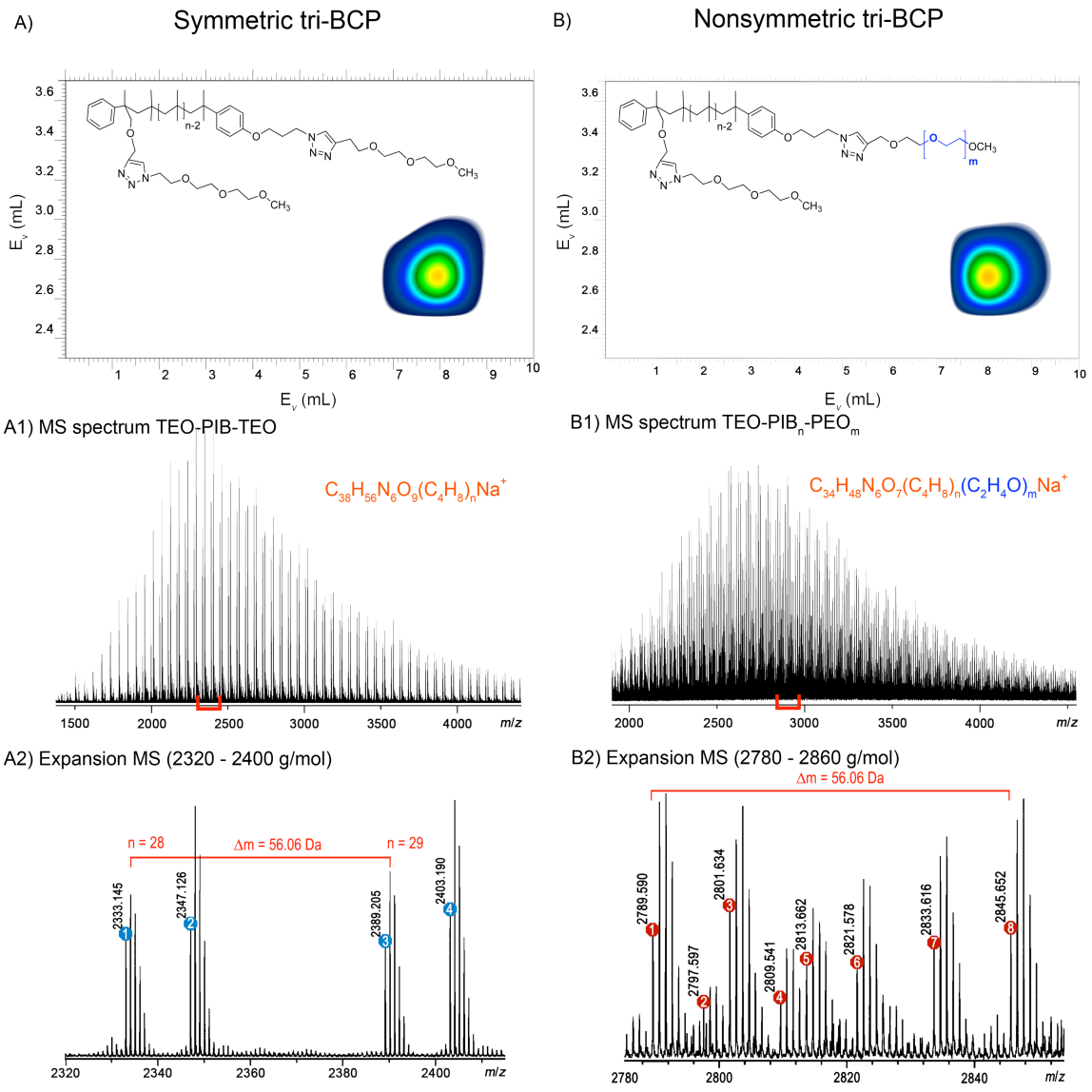

Figure 8. Two-dimensional liquid chromatography coupled to MALDI-TOF MS of symmetric and nonsymmetric PEO based PIB triblock copolymers (see publication Barqawi et al. *Macromolecules*, 2013, 46, 7638-7649). In (A and B) recorded 2D-chromatograms, (A1 and B1) full view of recorded MS spectra and (A2 and B2) magnified part of MS spectra demonstrating the repeating unit and all assigned species (compare **Table 5** and **6**).

Thus, a series of azide/alkyne "click"-reactions after living polymerization reactions were used to prepare either symmetric or nonsymmetric PIB-PEO based di- and triblock copolymers. In order to demonstrate the efficiency of the "click"-reaction and futhermore the purity of the final triblock copolymers, the critical conditions of the PIB-homopolymers (M_n = 3 – 30 kg mol^{-1}) in the isocratic elution mode (LCCC method) were determined.

Table 5. Experimental results of 2D-LCCC/SEC offline coupled to MALDI-TOF-MS of symmetric tri-BCP.

Entry	Polymer structure[1]	Species	m/z found [Da]	m/z simulated [Da]	Error [ppm]
1	**n = 28**	$[C_{150}H_{280}N_6O_9]\ Na^+$	2333.145	2333.153	6.1
2	n = 29[2]	$[C_{151}H_{282}N_6O_8]\ K^+$	2347.126	2347.148	9.3
3	**n = 29**	$[C_{154}H_{288}N_6O_9]\ Na^+$	2389.205	2389.216	6.0
4	n = 30[2]	$[C_{155}H_{290}N_6O_8]\ K^+$	2403.190	2403.210	8.4

[1] $\sum = C_{38}H_{56}N_6O_9(C_4H_8)_n$
[2] Fragment peak ($-C_2H_4OCH_3$)

As a consequence, the final triblock copolymers (tri-BCPs) were successfully separated from their intermediates using a reversed-phase Atlantis-RP C18 column as stationary phase and an eluent mixture of methyl-tert-butyl ether/methanol (85.34/14.66 (*w*/*w*)) as mobile phase. On the basis of the PEO segment length and overall hydrophobicity of the di- and tri-BCPs, a complete separation of the stepwise "click" products was observed, which allowed a quantitative analysis of their chemical composition.

Table 6. Experimental results of 2D-LCCC/SEC offline coupled to MALDI-TOF-MS of nonsymmetric tri-BCP.

Entry	Polymer structure[1]	species	m/z found [Da]	m/z simulated [Da]	Error [ppm]
1	**n = 33; m = 6**	$[C_{178}H_{336}N_6O_{13}]\ Na^+$	2789.590	2789.571	7.0
2	n = 30; m = 10	$[C_{174}H_{328}N_6O_{17}]\ Na^+$	2797.597	2797.488	6.5
3	n = 34; m = 5	$[C_{180}H_{340}N_6O_{12}]\ Na^+$	2801.634	2801.607	9.7
4	n = 31; m = 9	$[C_{176}H_{332}N_6O_{16}]\ Na^+$	2809.541	2809.524	5.9
5	n = 35; m = 4	$[C_{182}H_{344}N_6O_{11}]\ Na^+$	2813.662	2813.644	6.6
6	n = 32; m = 8	$[C_{178}H_{336}N_6O_{15}]\ Na^+$	2821.578	2821.561	6.3
7	n = 33; m = 7	$[C_{180}H_{340}N_6O_{14}]\ Na^+$	2833.616	2833.597	6.6
8	**n = 34; m = 6**	$[C_{182}H_{344}N_6O_{13}]\ Na^+$	2845.652	2845.633	6.5

[1] $\sum = C_{34}H_{48}N_6O_7(C_4H_8)_n(C_2H_4O)_m$

Finally, direct coupling of the 2D-LC/GPC (see **Figure 8**) to ESI- and/or MALDI-TOF MS allowed further a full identification of all reaction steps (see **Table 5** and **6**) proving the chemical structure of the final symmetric and nonsymmetric triblock copolymers, as demonstrated in **Figure 8** for a symmetric and nonsymmetric tri-BCP.

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation zum Thema *„Multidimensional Liquid Chromatography coupled to Soft Ionization Techniques: Analysis of Telechelic Polymers“* selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die den zitierten Werken wörtlich oder inhaltlich entnommenen Stellen als solche gekennzeichnet habe. Weiterhin versichere ich, dass die vorliegende Arbeit noch an keiner anderen Einrichtung zur Begutachtung vorgelegt wurde.

Halle (Saale), den 26.09.2014 .....

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Working Experience

Scientific fellow, Martin-Luther Universität Halle-Wittenberg

Halle, Sachsen-Anhalt — Sep. 2013 - present

Accomplishments

- Characterization of dendrimers and block copolymers via 2D-LC/SEC- (MALDI-TOF) and/or (ESI-TOF) MS.
- Structural elucidation via spectroscopic and spectrometric techniques.
- Supervising PhD students, analytics projects.

Scientific researcher, Martin-Luther-Universität Halle-Wittenberg

Halle, Sachsen-Anhalt — Oct. 2009 - Jun. 2013

Accomplishments

- Applying/developing hyphenated techniques towards the analysis of telechilic poly(e-caprolactone), polyisobutylene and diblock, -triblock copolymers.
- Coupling of various analytical techniques seeking for extensive information about complex mixtures of polymers.

Scientific researcher, Martin-Luther Universität Halle-Wittenberg

Halle, Sachsen-Anhalt — Aug. 2007 - Sept. 2009

Accomplishments

- Cooperation with Dynea Austria GmbH, project: enhancement and modification of melamine-formaldehyde resins towards scratch resistant ability.
- Cooperation with Dynea Austria GmbH, Project: Analysis of melamine-formaldehyde resins via hyphenated techniques, *LC-MALDI-TOF-MS*.

Senior technical officer, Fosroc International

Amman, Jordan/Dubai, UAE — April -2006 - Oct. 2006

Accomplishments

- Successfully lead a team of 25 members working on polyurea coating project at ABC/Aqaba.
- Optimized laboratory analysis/testing methods for concrete admixtures such as plasticizers and super-plasticizers.
- Created/modified new formulations for cement mix and concrete admixtures.

Scientific researcher, Pharmaceutical Research Unit

Amman, Jordan — Feb, 2006 - April, 2006

Accomplishments

- Bioequivalence studies on selected drugs using hyphenated techniques.
- Calibration and validation of the analytical instruments.

Scientific researcher, Degussa Construction Chemicals

Halle, Sachsen-Anhalt and Trostberg, Bayern — 2004-2005

Accomplishments

- Standardized new analytical methods for characterization of starch- and cellulose ethers
- Developed formulations of special polymers in use for cementitious mortars.

Education

Martin-Luther-Universität Halle-Wittenberg

Dr. rer. nat. — 2014 (April. expected)
Thesis topic

Multidimensional Liquid Chromatography Coupled To Soft Ionization Techniques: An Approach For Telechelic Polymer Analysis.

Martin-Luther-Universität Halle-Wittenberg

M.Sc. Applied Polymer Science— 2004 (Dec.)
Thesis topic

Studies on vinylation of substituted alcohols and urethanes.

Applied Science Private University Amman-Jordan

B.Sc. Applied Chemistry— 2002 (Feb.)

Major topics

Analytical chemistry, organometallics, industrial chemistry.

Language Proficiency

- English: excellent command spoken, read and written (TOEFL, GRE)
- German: excellent command spoken, read and written (verhandlungssicher)
- Swedish: moderate command spoken, read and written
- Spanish: basics
- Arabic: native

Computer Skills

- Office enterprise.
- Chemistry software, chemoffice, chemdraw, ISIS, NMR, IR.
- Engineering software: Ansys 5.4.
- Databases e.g. Scifinder.
- Web Design: adobe creative suite.
- Java, javascript, C++.
- Servers, networking.

Lab Skills

- Operator of GPC/MALDI-TOF-MS, LC/ESI-TOF-MS, and 2D-LC/SEC.

Publications

- Synthesis of an Amphiphilic Beta-Turn Mimetic Polymer-Conjugate, *ACS Macro Letters*, **2014**, 3, 393–397
- Facile Preparation of Supramolecular (ABAC)n Multiblock Copolymers from Hamilton Wedge and Barbiturate-Functionalized RAFT Agents, *Polym. Chem.*, ***2014***, *5, 2891-2900*
- 2D-LC/SEC-(MALDI-TOF)-MS Characterization of Symmetric and Nonsymmetric Biocompatible PEOm–PIB–PEOn Block Copolymers, *Macromolecules* 46(19): 7638-7649.
- Multidimensional characterization of alpha, omega-telechelic poly(-caprolactone)s via online coupling of 2D chromatographic methods (LC/SEC) and ESI-TOF/MALDI-TOF-MS, *Macromolecules* 45(24): 9779-9790.

- Azide/alkyne-“click”-reactions on amino resin materials: An LC-ESI-TOF analysis, *J. Polym. Sci., Part A: Polym. Chem.* 48(21): 4855-4866.
- Synthesis and Organization of Three-Arm-Star PIB-PEO Block Copolymers at the Air/Water Interface: Langmuir- and Langmuir-Blodgett Film Investigations, *Macromol. Chem. Phys.* 211(2): 204-214.
- Macrocyclization of polymers via ring-closing metathesis and azide/alkyne-“click”-reactions: An approach to cyclic polyisobutylenes, *J. Polym. Sci., Part A: Polym. Chem.* 48(3): 671-680.
- Cyclic alkoxyamine-initiator tethered by azide/alkyne-"click"-chemistry enabling ring-expansion vinyl polymerization providing macrocyclic polymers, *J. Polym. Sci., Part A: Polym. Chem.* 48, (15): 3402–3416.
- Monitoring Block-Copolymer Crossover-Chemistry in ROMP: Catalyst Evaluation via Mass-Spectrometry (MALDI), *Macromolecules 42* (24): 9457–9466.

Poster Contributions

- EUPOC, 2009, Gargnano Lago di Garda – Italy
- DGMS, 43. Jahrestagung der Deutschen Gesellschaft für Massenspektrometrie, 2010, Halle-Germany
- Euchem 3rd, 2010, Nuremberg – Germany
- SCM - 6, 2013, Dresden – Germany

Oral Contributions

13. Colloquium; „MALDI-TOF-MS and Synthetic Polymers, 2009, BAM, Berlin-Germany